COMPARATIVE EVALUATION OF SEALER PENETRATION INTO ROOT DENTIN FOLLOWING PRETREATMENT WITH TWO DIFFERENT CHELATING AGENTS – AN INVITRO STUDY

A Dissertation submitted
in partial fulfilment of the requirements
for the degree of

MASTER OF DENTAL SURGERY

BRANCH – IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY

CHENNAI- 600032

2015 – 2018

ADHIPARASAKTHI DENTAL COLLEGE AND HOSPITAL

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CERTIFICATE

This is to certify that **Dr. S. SARAVANAN**, Post Graduate student (2015-2018) in the Department of Conservative Dentistry and Endodontics, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319, has done this dissertation titled **"Comparative Evaluation of Sealer Penetration Into Root Dentin Following Pretreatment with Two Different Chelating Agents – An Invitro Study.** Under our direct guidance and supervision in partial fulfilment of the regulations laid down by the Tamilnadu Dr.M.G.R Medical University, Chennai – 600032 for MDS., (Branch-IV) **CONSERVATIVE DENTISTRY AND ENDODONTICS** degree examination.

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ACKNOWLEDGEMENT

I thank **Almighty God** for answering my prayers and making me what I am today.

I avail this opportunity to express my gratitude and reverence to my beloved Teacher **Dr. S. Thillainayagam, M.D.S**., Professor, Department of conservative dentistry and endodontics, Adhiparasakthi Dental College and Hospital Melmaruvathur. His pursuit for perfection and immense support were a source of constant inspiration to me. Words cannot express my gratitude for his quiet confidence in my ability to do the study, his willingness to help and clear the stumbling blocks along the way and his tremendous patience till the completion of this study.

A special mention of thanks to **Dr. Prabhakar joseph, M.D.S.,** professor and HOD, Department of Conservative Dentistry and Endodontics for valuable suggestions and encouragements throughout my completion of my Main dissertation.

It is my duty to express my thanks to my Co-guide **Dr. S. Karthikeyan, M.D.S.,** Reader for his valuable suggestions and encouragements throughout my completion of my Main dissertation.

I am thankful and express my gratitude to my previous teachers **Dr. S. Sathyakumar, MDS.,** Professor, **Dr. D. S. Dinesh, MDS.,** Professor, **Dr. Prasanth,** Professor for their excellent guidance, immense help and support for the initiation of this study.

I thank our Managing Director **Dr. T. Ramesh, MD**., for his vital support and allowing us to utilize all the facilities provided.

I am thankful to **Dr. S. Thillainayagam, M.D.S.,** our beloved Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur for providing us the opportunity to utilize the facilities of the college.

I am thankful to express my gratitude to my teachers **Dr. A. Jayasenthil, M.D.S.,** Reader, **Dr. N. Bharath, M.D.S.**, Reader, **Dr. N. Raghunathan, M.D.S.,** Senior lecturer, **Dr. M. Purushotham M.D.S.,** Senior lecturer **Dr. E. Premkumar M.D.S.,** Senior lecturer, and **Dr. P. Kaushalya, M.D.S.,** Senior lecturer.

I am extremely grateful to **Mr. Mohan Kumar** and **Mr. Anbu Dhayanidhi,** Head**,** Material Testing Lab, CIPET, Guindy, for granting me permission to conduct the study in their departments and helping me to bring out my study.

I would like to thank Statistician **Dr. Seenuvasan, MDS.,** Biostatistics, for helping me in statistical works.

I also wish to thank my Co-Pg **Dr. A. Karthikeyan**, **Dr. Yelamanchianusha** and my senior **Dr. C. Ravivarman**, **Dr. S. Srikanth** and I warmly acknowledge my juniors **Dr. Y. Reeja, Dr. K. Santhoshkumar, Dr. N. Mohankumar, Dr. A. Yoghapadhma, Dr. T. Vaibhavi,** and **Dr. V. Kanagapriyaa.**

I owe my gratitude to my father **D**. **Selvaraj,** Teacher and my mother **S. Kamatchi,** Teacher who stood beside me during my hard time and sacrificed so much to make me what I am today. A special thanks to my loving wife **Dr. P.M. Mamatha, MD (Paediatrics)** and my cute son **S. Mohana Balaji** and Daughter **S. Yogitha** for their sacrifice and encouragement throughout my career.

My acknowledgement wouldn’t be complete without mentioning my teaching and non-teaching staffs **Mrs. Mahalakshmi, Mrs. Kanaga, Mrs. Kamatchi, Mrs. Valli,** Office staffs, and Librarian **Mr. P.Maveeran**, **Mr. K.Selvakumar** and X-Ray Technicians **Mr. Prabhu**, **Mr. Moorthy** and Medical Record Department **Mr. Dhakshanamoorthy** for being my strength, by giving me continual support all the time.

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DECLARATION

TITLE OF THE DISSERTATION	**“Comparative Evaluation of Sealer Penetration Into Root Dentin Following Pretreatment With Two Different Chelating Agents – An Invitro Study”.**
PLACE OF THE STUDY	Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319
DURATION OF THE COURSE	3 years
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I hereby declare that no part of the dissertation will be utilized for gaining financial assistance or any promotion without obtaining prior permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319. In addition, I declare that no part of this work will be published either in print or in electronic media without the guides who has been actively involved in dissertation. The author has the right to reserve for publish work solely with the permission of the Principal, Adhiparasakthi Dental College and Hospital, Melmaruvathur – 603319

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Signature of the candidate

ABSTRACT

BACKGROUND: The success of root canal treatment depends on cleaning and shaping, followed by three-dimensional obturation of the root canal system to prevent reinfectiion. Tubular penetration and adaptation of sealer can determine the sealability of root filling which in turn are determined by many factors like smear layer removal, dentinal permeability, root canal dimension, and the physical and chemical properties of the sealer. Smear layer removal forms an important determining factor in sealer penetratbility. Traditionally, it is done with 5.25% NaOCl irrigation followed by 17 % EDTA. Since EDTA is not biodegradable and its possible damage to periapical tissues on extrusion search for alternative chelating agent is going on. Phytic acid, known as inositol hexakisphosphate(IP6), is a naturally occurring agent, has ability to chelate with positively charged multivalent cations while having minimal effect on pulpal cells we evaluated 1% phytic acid for its chelating ability.

AIM: The aim of the present in-vitro study was to evaluate and compare the penetration of AH Plus sealer into dentinal tubules by using scanning electron microscope following treatment with two different chelating agent i.e 17% EDTA and 1% phytic acid.

MATERIALS AND METHODS: Sixty freshly extracted human mandibular first premolars with single straight root canals were used in the study. These were randomly divided into three equal groups of 20 samples each. The crowns of all teeth were cut at Cemento-enamel junction using high speed tapering diamond under air water spray with remaining root length 12±1mm. The working length were established by placing a size 10 K file into each sample until the tip of the file was visible at the apex. Canal length was established 1 mm short of the apex. The root canals were prepared using the ProTaper rotary system to an apical size of F3, and apical patency was rechecked using size-10 K- file throughout the preparation. During the entire preparation, alternate irrigation and

recapitulation was done with 5.25% sodium hypochlorite (NaOCl) (Avarice Laboratory, Ghaziabad, India) and #10 K-file, respectively.

Samples were divided into 3 groups(A,B,C) with20 samples each.

1. Group A (EDTA): samples(n=20) were irrigated with 10ml of 17% EDTA for 1min.
2. Group B (1% Phytic acid): samples(n=20) were irrrigated with 10ml of 1%phytic acid for 1min.
3. Group C (distilled water): samples(n=20) were irrigated with 10ml distilled water for 1min.

After irrigation with different irrigating agents all root canals were obturated with help of F3 Size GP and AH-Plus sealer. Samples were then sectioned in the bucco-lingual direction with the help of sorensen disc Smear layer produced during sectioning were removed by cleansing with 17% EDTA and 3% Naocl. Samples were studied for dentinal tubule penetration at all the three levels - coronal, middle and apical levels. The penetration of sealer into the dentinal tubules were assessed by using scanning electron microscopic(SEM) examination.

RESULTS: It was found that highest sealer penetration depth was found in group A (EDTA) followed by group B least with group C. Though the penetration in group B was lower than group A it had significant levels of penetration indicating a potential chelating effect. In all three groups coronal region had highest levels of penetration followed by middle region and least in apical regions.

CONCLUSIONS: Within the limitations of the study it can be concluded that EDTA group had highest sealer penetration. Phytic acid group had intermediate effect indicating potential chelating ability. Further studies are essential to confirm required concentration, pH, exposure time for its optimal chelating effect for using it as an alternative chelating agent.

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LIST OF ABBREVIATIONS

NaOCl	:	Sodium hypochlorite
EDTA	:	Ethylene diamine tetra acetic acid
SL	:	Smear layer
SEM	:	Scanning electron microscope
ISO	:	Indian standard Organization
WL	:	Working length
IP6	:	Inositol Hexakisphosphate
PA	:	Phosphoric acid
ALP	:	Alkaline phosphatase
µm	:	Micrometer
MIC	:	Minimum Inhibitory Concentration
NiTi	:	Nickel Titanium
GP	:	Gutta percha

INTRODUCTION

The success of root canal treatment depends on cleaning and shaping, followed by three-dimensional obturation of the root canal system. The mechanical instrumentation of the root canal produces the amorphous irregular smear layer (SL) containing inorganic debris, organic materials like pulp tissue, odontoblastic processes, necrotic debris, microorganisms and their metabolic byproducts.[1] McComb and Smith were the first investigators who showed the presence of a SL in instrumented root canals.[2] Despite the controversies regarding removal of the SL, most clinicians have concluded that its presence contributes to leakage and compromises the seal of the root canal filling.[3,4] It can also serve as a source of nutrients for microorganisms[5,6]. In a systematic review Shahravan by *et al*[7], it was concluded that SL removal improves the fluid-tight seal of the root canal system, as suggested by most authors.

Chelating agents are used in endodontics to soften the dentin, facilitating access to the entire root canal length and to remove the smear layer formed during root canal instrumentation. In 1893, Callahan suggested the use of sulphuric acid (25-50%) to gain access to narrow calcified root canals.[8] Other acids such as hydrochloric and nitric acids have also been tried. Demineralisation of hard tissues by organic chelating agents at natural pH was reported in 1953 by Nikiforuk and Sreebny[9] who noted that calcium chelated above a pH of

6 and the upper limit of chelation was at pH 7.5.The origin of these agents dates back to 1951,when the first report on the demineralising effect of EDTA on dental hard tissue was published.[10] Chelators were first introduced into endodontics by Nygaard –Ostby[11] in 1957,who recommended the use of a 15% EDTA solution (pH7.3) with the following composition:

- Disodium salt of EDTA(17 g)
- Aqua dust(100 ml)
- 5M sodium hydroxide(9.25 ml)

To increase the cleaning and bactericidal activity of EDTA solution, a detergent was added. This new composition was known as EDTAC by VonderFehr and Nygaard-Ostby in 1963.[12] EDTAC is produced when EDTA is mixed with 0.84g of a quarternary ammonium compound. This new composition had properties like reducing surface tension of the irrigant, helps in wetting of the entire root canal wall and hence increases the ability of chelators to penetrate the dentine. Initially, chelators were used as liquid for irrigation during mechanical instrumentation of root canal system. In 1969, Stewart et al[13] presented RC-Prep (premier Dental, Plymouth Meeting, PA, USA)probably the best known paste type chelating agent .In 1978,Kaufman et al[14] presented Salvizol (Ravensburg, Konstanz, Germany) containing 5% aminoquinaldinumdiacetate in propylene glycol with a pH of 6.6. Scelza et al[15] in 2000 introduced EDTA-T ,consisting of 17% EDTA and sodium laurylether sulphate (Tergentol).

Different methods, irrigating solutions and chelators have been used to remove the smear layer. Currently, the subsequent use of 17% ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) is the recommended regiment and gold standard for removal of the inorganic and organic components of the smear layer, respectively.

EDTA has been the most commonly used irrigant for this purpose since 1957[11] in a concentration of 17% and an application time of 1–5 minutes.[16] It is most commonly synthesized on an industrial scale from ethylenediamine, formaldehyde and sodium cyanide. This method results in the formation of impurities that are detrimental to most applications of this chelating agent.[17] This synthetic persistent material is being overused and is considered one of the major organic pollutants discharged in water.[18] Because EDTA is not readily biodegradable, there have been some concerns about the leakage of this irrigant into the periapical tissue. Because of these concerns, the extrusion of EDTA beyond the root canal should be avoided.[19,20] Considering these facts, an alternative agent for smear layer removal is warranted, and the search for more biocompatible material to replace EDTA is still going on.

Phytic acid

Phytic acid, known as inositol hexakisphosphate (IP6), is the major storage form of phosphorus in plant seeds.[21] Phytic acid is reported to inhibit the intestinal absorption of some minerals such as calcium (Ca2+), zinc, iron, and magnesium.[22] As a result of its strong negative charge , it has the ability to chelate with positively charged multivalent cations, forming complexes that are soluble under acidic conditions but precipitate at neutral pH.[23] Recently, phytate (a salt of IP6) has been shown to have a protective role in preventing osteoporosis through decreasing the solubility of calcium salts.[24] This natural reagent has been reported to have a cross-linking effect on protein nanofibers that are used for cardiac tissue engineering.[25] Literature on the use of IP6 in dentistry is scarce; however, IP6 has been postulated to have anticariogenic or cariostatic effects through the reduction of enamel solubility[26] or through its high affinity to hydroxyapatite, thus reducing the adsorption of bacteria to tooth surfaces (antiplaque effect).[27] Based on the ability of this naturally occurring agent to chelate Ca2+, form complexes with minerals, and/or cross-link collagen,[28] it can be used as an alternative with minimal effect on pulpal cells. Hence, the aim of this study is to determine the efficacy of the phytic acid as an alternative chelating agent which helps in sealer penetration into root dentin.

The main objective of a root canal filling is to seal the root canal system to prevent reinfection.[29] Normally, a root canal filling is associated with a hard core, like gutta-percha, and a sealer to better adapt the root canal filling material and complete the seal of the root canal filling in the most effectual manner.[30] Therefore, the sealer root canal wall interface is crucial for the sealing of the root canal system. The sealer can fill the irregularities of the root canal wall and the dentinal tubules, which cannot be filled by gutta-percha. Increase in contact surface between the filling material and dentin, leads to better sealing ability and hence, better sealer penetration into tubules.[31] Sealers also have antimicrobial effect in tubules which increases with better penetrability.[29,32]

AH Plus sealer

Among the root canal sealers used, AH Plus has shown to have better adaptation to the root canal wall, tubular penetration, and adaptation to the peritubular dentine directly. The findings for AH Plus are supported by other studies.[33,34] AH Plus consists of a paste-paste system, which is delivered in two tubes and in a new double barrel syringe. The epoxide paste consists of radio opaque fillers, Aerosil and diepoxide. The amine paste consists of three different types of amines, radio opaque fillers and Aerosil. AH Plus is characterised by very good mechanical properties,[35] high radio opacity, little polymerisation shrinkage, low solubility, and, not least, a high degree of stability on storage.

AIM AND OBJECTIVES

The aim of the present in-vitro study was to evaluate and compare the penetration of AH Plus sealer into dentinal tubules by using scanning electron microscope following treatment with two different chelating agents.

OBJECTIVES

The objective of present in-vitro study was to evaluate and compare the penetrability of AH plus sealer into the dentinal tubules following pretreatment with two different chelating agents namely 17% EDTA solution and 1%phytic acid solution.

Penetrability of sealers into dentinal tubules was assessed using scanning electron microscope in micrometers.

REVIEW OF LITERATURE

Goldberg F et al (1977)[38] did a study on analysis of the effect of EDTAC on dentinal walls of root canal. The results suggested that the use of EDTAC as an aid in the biomechanical preparation of the root canal helps in the cleaning and disinfection of the dentinal wall by eliminating most of the superficial layer of the dentinal shavings and material loosened principally during instrumentation. It also facilitated the action of drugs by increasing the diameter of the dentinal tubules. It was also suggested that the use of EDTAC conditions the dentinal walls of the root canal to provide greater adhesion of the obturating material. This is due to the ability of EDTAC to increase the permeability of dentin.

Yamada RS et al (1983)[39] did a study on efficacy of instrumenting the root canal with 1ml of 5.25% NaOCl solution between each instrument and final flushing with 20ml of various solutions or combinations of solutions and the scanning electron microscopy showed that a final flush with 10ml of 17% EDTA buffered to pH 7.7 followed by 10ml of 5.25% NaOCl solution was the most effective. This is due to the combined use of a tissue solvent with a chelating agent.

Berg MS et al (1986)[40] did a scanning electron microscopic study on comparison of five irrigating solutions namely Salvizol, NaOCl, Gly-Oxide in combination with NaOCl, REDTA and sterile saline. The results suggested that when REDTA solution is used during biomechanical preparation, cleaner root canals are attained. The cleansing property of REDTA was considered to be superior due to the differencein pH.

Cergneux M et al (1987)[41] did a study to evaluate the influence of the smear layer on the sealing ability of canal obturation which had previously been cleaned chemically by EDTA or mechanically by ultrasound. The canals were prepared under irrigat ion with NaOCl and specimens were divided into control group, ultrasound group and EDTA group and the specimens were subjected to dye infiltration before being sectioned at various levels from apex. The results showed some differences in leakage between the three groups at levels close to the apex. EDTA treated canals showed the least infiltration, while those treated with ultrasound showed significantly less compared with the control group. The reason maybe that the ultrasound procedures employed were less effective in eliminating the smear layer from the canal walls.

Berutti E et al (1997)[42] did a study to evaluate the penetration ability of different irrigants into dentinal tubules and histological examinations showed that group which used 10% EDTA, Triton and 5% NaOCl showed an infection-free area of tubules to a mean depth of 130 μm, below this was an infected area of variable extent. It corresponds to the extreme penetration of the tubular infection. In some specimens the dentinal tubules were perfectly clean and bacteria free from their entire length. This is due to the regularity and larger size of the dentinal tubules in this area.

Saleh AA et al (1999)[43] evaluated the effect of endodontic irrigation solutions on microhardness of root canal dentine. The irrigant solutions used were 3% H_2O_2 , 5% NaOCl and 17% EDTA. The results showed that, irrigation with either H_2O_2/NaOCl or EDTA decreased the microhardness value of root dentin and EDTA reduced the hardness more than H_2O_2/NaOCl irrigation. The observations of the study suggest that canal irrigation with various chemical solutions leads to structural changes as evidenced by the reduction of dentin microhardness. The chelating action of the EDTA solution induces an adverse softening potential on the calcified components of dentine, which results in subsequent reduction in the microhardness.

Choudary M et al (2000)[44] did a study on effect of EDTA, NaOCl and their combination at different time periods namely 5, 10, 15, 20, 25 and 30 minutes on smear layer and suggested that their combined use kept for a total of 20 minutes removed the smear layer completely. The chelating effect of EDTA demineralised and removed the inorganic component of smear layer and NaOCl used over the dentin dissolved the organic material.

Calt S et al (2002)[45] did a study to evaluate the effects of EDTA on smear layer removal and on the structure of dentin after 1 and 10 minutes of application. The results showed that 1 min EDTA irrigation is effective in removing the smear layer. However a 10 minutes application of EDTA caused excessive peritubular and intertubular dentinal erosion. And the study suggested that the procedure of EDTA application in root canals should not be prolonged more than 1 minute during endodontic treatment.

Torabinejad M et al (2002)[46] did a review on the evidences regarding clinical implications of smear layer in endodontics. On the basis of the available evidence, it was concluded that current methods of root canal instrumentation produce a layer of organic and inorganic material that may also contain bacteria and their byproducts. This layer covers the instrumented walls and mayprevent the penetration of intracanal medications into the dentinal tubules and may affect close adaptation between root canal filling materials and the root canal walls.

Schafer E et al (2003)[47] did a study to compare the weight loss of eight different root canal sealers in water and in artificial saliva with different pH values. The tested sealers were epoxy resin sealer, calcium hydroxide sealer, zinc oxide eugenol sealer, glass ionomer sealer and polyketone based sealers. The samples were immersed in double distilled water or artificial saliva with different pH values of 7.0, 5.7 and 4.5. Mean weight loss was then evaluated. It was found that even after 28 days of storage in water, AH 26, AH Plus. RSA RoekoSeal and Diaket showed less than 3% weight loss. And it was concluded that AH Plus showed the least weight loss of all sealers tested.

S. Sevimay & D. Dalat et al (2003)[48] conducted a study on penetration and adaptation of three different sealers and concluded that AH 26 was the best sealer penetrating into dentinal tubules and adapted to dentinal walls when compared with the CRCS and RSA.

Ari H et al (2004)[49] evaluated the effects of endodontic irrigants on the microhardness and roughness of root canal dentin. The study evaluated the effect of 0.2% chlorhexidine gluconate, 5.25% NaOCl, 2.5% NaOCl, 3% H2O2, 17% EDTA and distilled water on microhardness and roughness of root canal dentin. In this study, the irrigant solutions were applied on root canal dentin surface for 15 min, and the surface microhardness and roughness tests were used to determine changes on dentin surface. Although 3% H2O2 and 0.2% chlorhexidine gluconate had no effect on surface roughness of root canal dentin, a significant increase on surface roughness was found in 2.5%, 5.25% NaOCl and 17% EDTA treated groups. According to the results of this study, 0.2% chlorhexidine gluconate appears to be an appropriate irrigation solution because of its harmless effect on the microhardness and roughness of root canal dentin.

A Khademi et al (2004)[50] did an *in vitro* study to determine the effect of EDTA and citric acid on smear layer removal in different regions of root canals. They concluded that use of both 17% EDTA and 7% citric acid offer desired results and they can remove smear layer from narrow and curved canals especially from apical region.

S. Sevimay et al (2005)[51] conducted a study on apical sealing ability and adaptation to dentine of two resin-based sealers and concluded that AH plus sealer has better apical sealing ability and adaptation to dentine than EndoRez sealer.

Stephen Cohen et al (2006)[52] stated that the most important objective is to fill the canal system completely and densely and to seal the apical foramina hermetically. Filling of root canal would be difficult if it were not designed and prepared specifically for use with gutta-percha cone. Further studies are being carried out by using heat or solvents to better adapt the gutta-percha to the canal space.

Dotto SR et al (2007)[53] did a study to compare the efficacy of 1% NaOCl, 24% EDTA gel and 17% EDTA solution in cleaning dentin walls after root canal instrumentation and it was suggested from the results that 1% NaOCl alone does not remove the smear layer and 17% EDTA solution and 24% EDTA gel used in association with 1% NaOCl were more effective.

Christian Ralf Gernhardt et al (2007)[54] evaluated Apical sealing ability of 2 epoxy resin–based sealers used with root canal obturation techniques based on warm gutta-percha compared to cold lateral condensation and concluded that the apical sealing ability of EndoRez is not as effective as that of AH Plus. Thermafilobturators and warm vertical condensation achieved seals with low dye penetration depth. The use of these techniques might decrease the risk of apical leakage.

Mamootil K et al (2007)[55] evaluated penetration of dentinal tubules by endodontic sealer cements in extracted teeth and cocluded that the depth and consistency of dentinal tubule penetration of sealers appears to be influenced by the chemical and physical characteristics of the materials. AH 26 resin-based sealers displayed deeper and more consisitent penetration than endorez and pulp canal sealer might be due to higher flow rate and low film thickness.

John I Ingle et al (2008)[56] stated that preliminary objectives of operative endodontics are total debridement of the pulpal space, development of a fluid –tight seal at the apical foramen, and total obliteration of the root canal. In addition micro leakage around coronal restoratios, down through the root canal filling, and out the apical foramen into the peri-radicular tissues is also a potential source of bacterial infestation.

Ajwani P et al (2010)[57] did a study on influence of smear layer on dentinal tubule penetration depth by different root canal sealers. He did the study with Endoseal, Apexit and AH Plus and he found out that smear layer removal allowed the penetration of all sealers to occur to a varying depth with Apexit and AH Plus penetrating statistically significantly deeper than Endoseal.

Onay EO et al (2010)[58] did a study to evaluate the sealing ability of 2 different resin-based endodontic filling systems after removal of smear layer with 2 different techniques. The samples were instrumented using HERO shaper rotary instruments. The canals were irrigated using 2.5% NaOCl between each instrument. In 2 groups extra rinse with 17% EDTA was done. Other 2 groups received irradiation with Er,Cr:YSGG laser. Apical leakage was measured with computerized fluid filtration meter at 1 and 4 weeks. Results showed that Er,Cr:YSG laser treatment did not enhance the sealing ability of the sealers compared with EDTA application.

Raid F et al (2010)[59] evaluated Shear Bond Strength Measurement of Three Different Adhesive Sealers to Dentin & Gutta-percha and concluded that the bonding system & the dentin pretreatment increased the adhesive potential of the AH26 sealer which had higher shear bond strength than the two glass -ionomer based sealers.

Bernardes et al (2010)[60] evaluated a study on evaluation of the flow rate of sealer 26, AH Plu, amd MTA obtura sealers and concluded that AH plus showed significantly flow rate compared with sealer 26 and MTA obtura due to epoxic resin incorporation. Calcium hydroxide component of sealer 26 makes it inferior to the AH plus and equivalent to MTA obtura.

Balguerie E et al (2011)[61] assessed the tubular adaptation, penetration depth and adaptation to the root canal walls in the apical, middle and coronal third of the root canal of 5 different sealers used in combination with softened gutta -percha cones. The 5 different sealers used along with gutta -percha are Acroseal, Endobtur, Ketac-Endo, RSA and AHPlus. It was observed that the AH Plus showed the best adaptation to the root canal wall, tubular penetration and adaptation to the peritubular dentin, followed by Acroseal. The reason is that epoxy resin sealers like AH Plus shows higher adhesion to the dentin and gutta percha and the flow of AH Plus is significantly higher than other sealers tested.

Neelakantan et al (2011)[62] conducted a study on The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer and concluded that AH Plus appears to bond to the organic phase of dentine.

S Anil Kumar et al (2011)[63] conducted a study on Comparative evaluation of the apical sealing ability and adaptation to dentine of three resin-based sealers and concluded that Epiphany sealer has a better apical sealing ability and adaptation to dentine than the AH Plus and Endorez sealers.

N Shokouhinejad et al (2011)[64] measured the average depth of dentinal tubule sealer penetration in the middle third of teeth obturated with gutta-percha/AH Plus (Dentsply, DeTrey, Konstanz, Germany), Resilon/Epiphany (Pentron Clinical Technologies, Wallingford,CT), and Resilon/Epiphany self-etch (SE) using scanning electron microscopy (SEM). It was concluded that the average penetration for Epiphany into dentinal tubules within the middle third of the roots was significantly deeper than that of Epiphany SE and AH Plus.

Gabriela Alexandra et al (2012)[65] conducted a study on Physicochemical properties of endodontic sealers of different bases and concluded that Ah plus, apexit plus and endofill sealers are in accordance with ANSI/ADA standards.

Rupali Chadha et al (2012)[66] has evaluated the depth of penetration of three resin based root canal sealers into dentinal tubules and found that penetration depth of Endo REZ and Epiphany into dentinal tubules is significantly greater than that of AH Plus.

Chandra Vijay Singh et al (2012)[67] in their study examined the in-vitro penetration depth of AH Plus, Resinoseal and zincoxideeugenol sealer into the dentinal tubules after removing smear layer by passive ultrasonic irrigation and found that AH Plus had maximum penetration depth.

Romel Joseph, Shishir Singh (2012)[68] conducted a study on Apical Sealing Ability of Four Different Sealers using Centrifuging Dye Penetration Method and concluded that AH Plus showed the least leakage compared to AH 26, Sealapex and Endoflas FS.

Nassar M et al (2013)[69] evaluated the effect of phytic acid(IP6), used as etchant, on resin-dentin bond strength, smear layer removal, and the viability of pulpal cells. The results demonstrated that all application times of IP6 produced bond-strength values that were significantly higher than that of the control. Phytic acid effectively removed the smear layer and plugs, thus exposing the collagen network. Phytic acid had a minimal effect on pulpal cells, whereas PA resulted in a marked decrease in their viability.

Aranda Garcia et al (2013)[70] evaluated effect of the root canal final rinse of 17% EDTA, Q mix, smear clear and water on the debris, smear layer removal and on the push-out bond strength of AH plus sealer and found that the efficacy of 17% EDTA, Q mix, smear clear was superior to the control groupn(water) in all the aspects. The ability to remove the debris and smear layer by smear clear and Q mix was as effective as that of 17% EDTA. The final rinse with17% EDTA has achieved highest push-out bond strength values.

Muliyar S et al (2014)[71] reviewed micro-leakage in endodontics and concluded that ah Plus with gutta-percha and epiphany with resilon provided the same coronal seal, whereas Epiphany with Resilon provided the best apical seal and aids in prevention of apical periodontitis or the retention of a functional tooth.

Johannes Ebert et al (2014)[72] conducted a study on Sealing ability of different versions of gutta-flow2 in comparison to gutta-flow and Ah plus and concluded that both forms of gutta-flow2 showed very good and predictable sealing ability when compared with the former versions of gutta-flow as well as with the established sealer Ah plus.

Daniel K (2014)[73] assessed the penetrability of two endodontic sealers(AH Plus and MTA Fillapex) into dentinal tubules, submitted to endodontic treatment and subsequently to endodontic retreatment. It was concluded that sealer penetrability is high during endodontic treatment. However, MTA Fillapex and AH Plus do not penetrate into dentinal tubules after endodontic retreatment.

H Ashraf et al (2014)[74] did an *in vitro* study to evaluate the ability of 17% ethylenediaminetetraacetic acid (EDTA), 18% etidronate and Er:

YAG on effective removal of the SL. The results showed statistically significant differences in terms of SL removal among the groups ($P<0.05$). The amount of removed SL by EDTA was significantly greater followed by Er: YAG laser and 18% etidronate. They concluded that within the limitations of this study, EDTA was more effective in removing SL compared to Er: YAG and etidronate

Nasser et al (2015)[75] investigated the effect of phytic acid inositol hexakisphosphate(IP6) as a final rinse on the surface of instrumented root canals and smear layered flat dentin surfaces treated with NaOCl and evaluated its effect on viability and alkaline phosphatase activity of osteoblast-like cells(MC3T3-E1). The results demonstrated the ability of IP6 to remove the smear layer from instrumented root canals and flat coronal dentin surfaces.when compared with EDTA, IP6 was less cytotoxic and did not affect the differentiation of MC3T3-E1 cells and concluded that IP6 had the potential to be an effective and biocompatible chelating agent.

K Kong et al (2015)[76] studied the effect of phytic acid(IP6) in stabilizing the morphology of dentine collagen network and resin-dentine bonding.IP6 demineralized dentine showed significantly higher ultimate tensile strength(UTS), when compare to phosphoric acid(PA) demineralized dentine. 5% glutaraldehyde(GA) and IP6 significantly improved UTS of PA-demineralized dentine. Field emission scanning electron microscope observation revealed that dentine collagen network was preserved by GA and IP6. No significant difference in µ tensile bond strength was found between the wet and dry IP6-etched dentine groups. They concluded that IP6 etching showed a structural stabilizing effect on demineralized dentine matrix an produced good resin-dentine bonding, regardless of dentine moistness or dryness.

Silva et al (2015)[77] evaluated the filling effectiveness and dentinal penetration MTA fillapex, AH plus and pulp canal sealer using stereo and confocal laser microscopy, concluded that presence of space voids in the filling material, MTA Fillapex was found to be inferior at 4mm and 6mm from the root apex. This behavior is probably because of the high solubility of MTA fillapex. Higher penetration of AH plus at 4mm from root apex might be due to presence of silver crystals provides excellent radiopacity but on the other hand it can obliterate the opening of dentinal tubules, compromising the intra-tubular sealer penetration.

R Nassar et al (2016)[78] studied the in vitro antibacterial effectiveness of used irrigants: sodium hypochlorite (NaOCl), EDTA, Phosphoric acid(PA) and chlorhexidine(CHX). They concluded that within the limitation of the study, despite that IP6 showed the smallest zone of inhibition in agar diffusion test, the results of MIC and MBC indicated that IP6 exhibits in vitro antibacterial effect against E.faecalis at low concentrations.

Nikhil V et al (2016)[79] evaluated the effect of phytic acid, EDTA, and chitosan solutions on the microhardness of human radicular dentin. They concluded that all tested chelating solutions reduced microhardness of the radicular dentin layer at all the levels. However, reduction was least at the apical level. EDTA caused more reduction in dentin microhardness than chitosan while phytic acid reduced the least.

Khader et al (2016)[80] conducted a study on dentinal tubular penetration depth of three root canal sealers and concluded that tubli-seal shows less depth of penetration as compared .to apexit plus and AH plus.

Akcay et al (2016)[81] conducted a study on dentinal tubule penetration of AH plua, iRoot SP, MTA fillapex, and gutta-flow bioseal root canalsealers after different final irrigation procedures and concluded dentinal tubule penetration area was significantly affected by the selection of root canal sealer, final irrigation procedure, and root canal third. Use of iRoot with PIP Stip or PUI seems advantageous in dentinal tubule penetration.

K kong et al (2016)[82] compared the etching effect of IP6 with phosphoric acid (PA) and ethylenediaminetetraacetic acid (EDTA) on resin–dentin bond strength, micromorphology of the etched dentin surface and nano leakage formation along resin–dentin interfaces and compared the protecting effect against collagen degradation. They concluded that IP6 effectively removed the smear layer and etched dentin, providing high bond strength values and causing minimal nano leakage and slight collagen degradation.

Saketh et al (2017)[83] evaluated the chealting and antimicrobial ability of phytic acid alone and in combination with different irrigating solutions. Chelating ability was assessed by calcium titration method and antimicrobial efficacy was assessed by agar diffusion method. In this study the chelating ability of phytic acid in combination with sodium hypochlorite gave the best result when compared to phytic acid alone. Phytic acid also showed more zone of inhibition indicating its antimicrobial efficacy is more compared with other irrigants. However the antimicrobial efficacy of phytic acid with other irrigants gave better results when compared to individual irrigants.

MATERIALS AND METHODS

MATERIALS

ARMAMENTARIUM:

1. Airotor – NSK T112002
2. Diamond Disc – BCR123-CR1Y4
3. ISO 10 - 30 size K – Files ----- Mani fies
4. 5.25% NaOCl – Avarice Laboratory, Ghaziabad, India
5. 17% EDTA – Anabond Stedman Pharma Research(P) Ltd, Kanchipuram, India
6. ProTaper NITI files – Dentsply Maillafer, Ballaigues, Switzerland
7. AH plus – Dentsply Maillafer, Ballaigues, Switzerland
8. Phytic acid- Tokyo Chemical Industry
9. Scanning Electron Microscope – 3.999999.0 S, ZIESS, Germany.

Sixty freshly extracted human mandibular first premolars with single straight root canals were used in the study. These were randomly divided into three equal groups of 20 samples each. All teeth were stored in 10% ethyl alcohol solution until the sample completion was completed.

Organic debris from the outer surface of the tooth was removed by immersing the teeth in 1% NaOCl solution for 4 days before starting of the experiment and subsequently placed in saline solution until they were used. The crowns of all teeth were cut at cemento-enamel junction using high speed tapering diamond under air water spray with remaining root length 12±1mm.

The working length were established by placing a size 10 K file (Kerr, Romulus, MI, USA) into each sample until the tip of the file was visible at the apex. Canal length was established 1 mm short of the apex. The root canals were prepared using the ProTaper rotary system (Dentsply Maillafer, Ballaigues, Switzerland), to an apical size of F3, and apical patency was rechecked using size-10 K- file throughout the preparation.

During the entire preparation, alternate irrigation and recapitulation was done with 5.25% sodium hypochlorite (NaOCl) (Avarice Laboratory, Ghaziabad, India) and #10 K-file, respectively.

Samples were divided into 3 groups(A,B,C) with20 samples each.

1. Group A (EDTA): samples(n=20) were irrigated with 10ml of 17% EDTA for 1min.
2. Group B (1% Phytic acid): samples(n=20) were irrrigated with 10ml of 1%phytic acid for 1min.
3. Group C (distilled water): samples(n=20) were irrigated with 10ml distilled water for 1min.

After irrigation with different irrigating agents all root canals were obturated with help of F3 size GP and AH-Plus sealer. The root canal sealer was mixed according to the manufacturer's directions. In all groups, gutta-percha was removed from the coronal 3 mm of all obturated root canals with a heated instrument and the coronal access cavities were sealed with Cavit.

Samples were kept at 37˚C for 1 week in 100% humidity to ensure complete setting of the sealer. Samples were then sectioned in the bucco-lingual direction with the help of sorensen disc Smear layer produced during sectioning were removed by cleansing with 17% EDTA and 3% Naocl. Samples were studied for dentinal tubule penetration at all the three levels - coronal, middle and apical levels. Samples were dehydrated by immersing them in 100% dry acetone for about one hour and will be subjected to vaccum drying in a vaccum oven for 3-4hours at 50˚C. the penetration of sealer into the dentinal tubules were assessed by using scanning electron microscopic(SEM) examination.

60 mandibular premolars

↓

Stored in saline solution

↓

Samples kept in 2% NaOCl solution for 4 days

↓

Decoronation done below Cemento-enamel junction

↓

samples were divided into three groups

root canal irrigation done with

group A:n=20	group B:n=20	group C: n=20
17% EDTA 10ml for one minute	1% phytic acid 10ml for one minute	distilled water 10ml for one minute

↓

Samples were kept at 37˚C for 1 week

↓

Sections made at Cervical, Middle, Apical

↓

Scanning Electron Microscopic Examination Done

Fig 1: Samples collection for assessment of sealer penetration

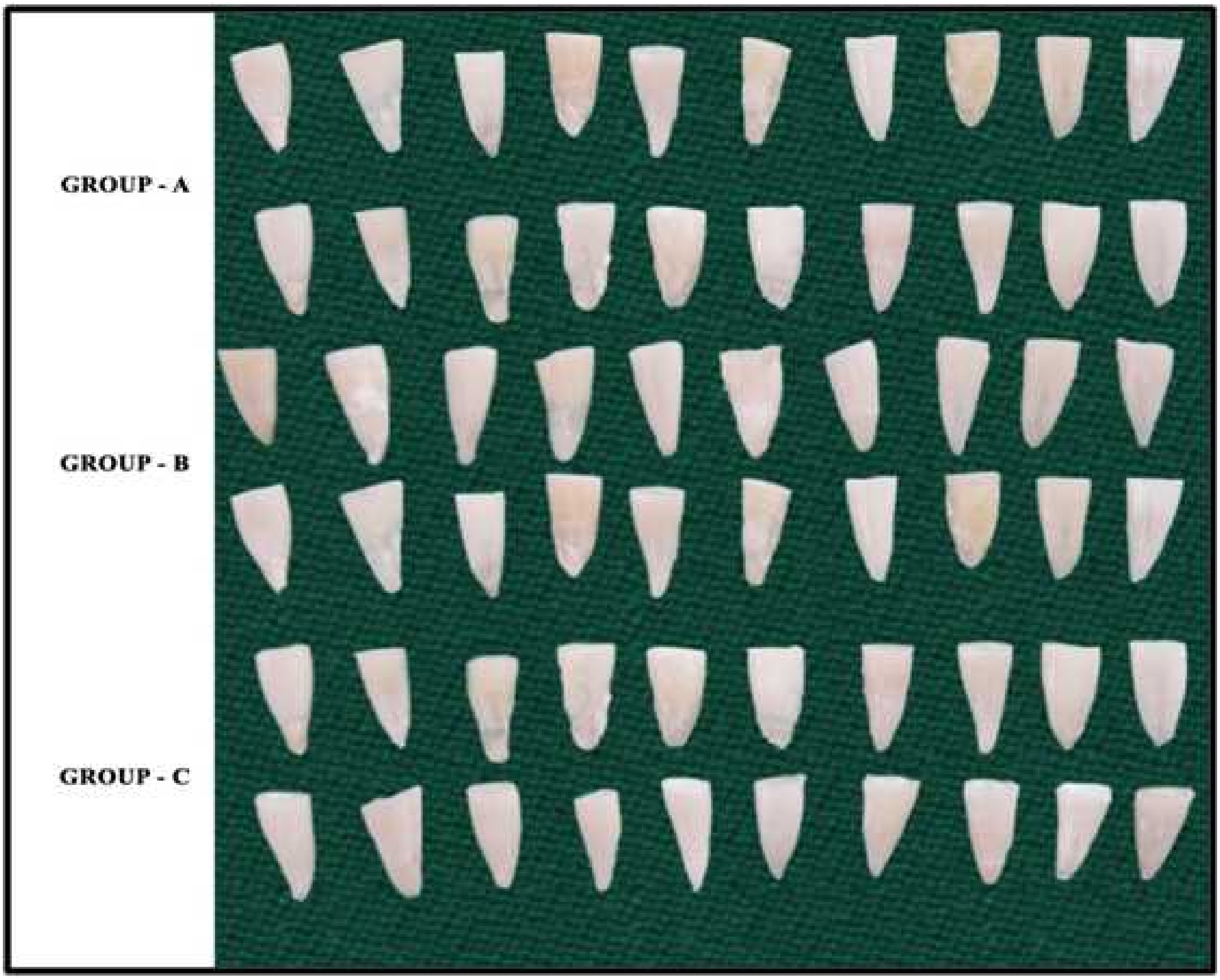


Fig 2: Decoronated sample specimens

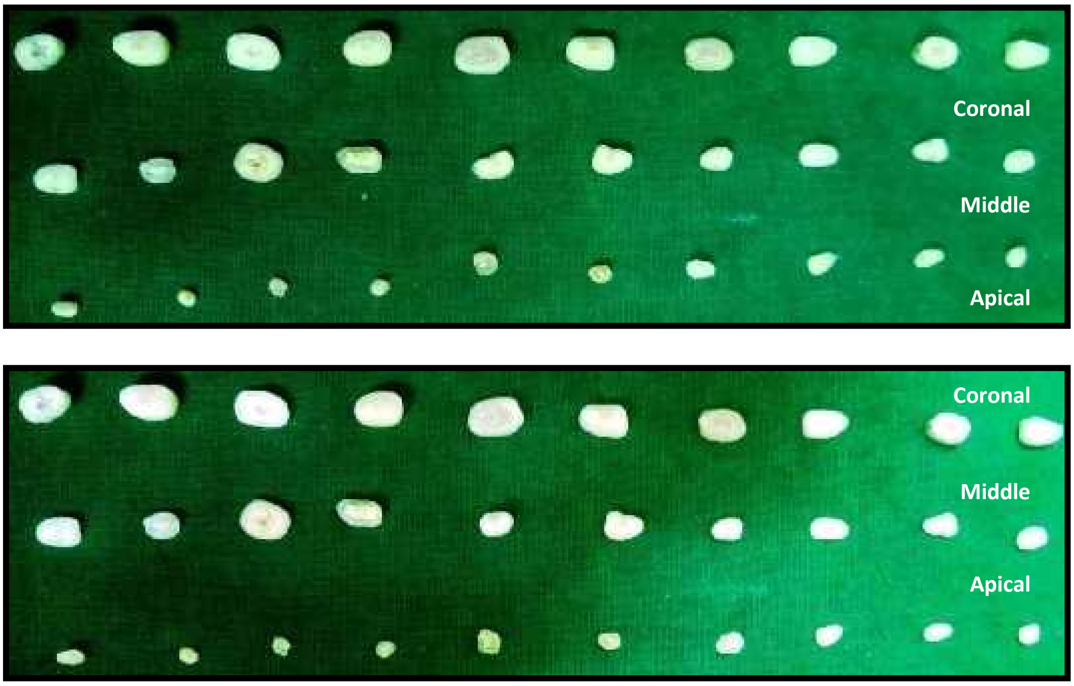


Fig 3. Transverse section of obturated samples for evaluation of sealer penetration in Group A(EDTA)

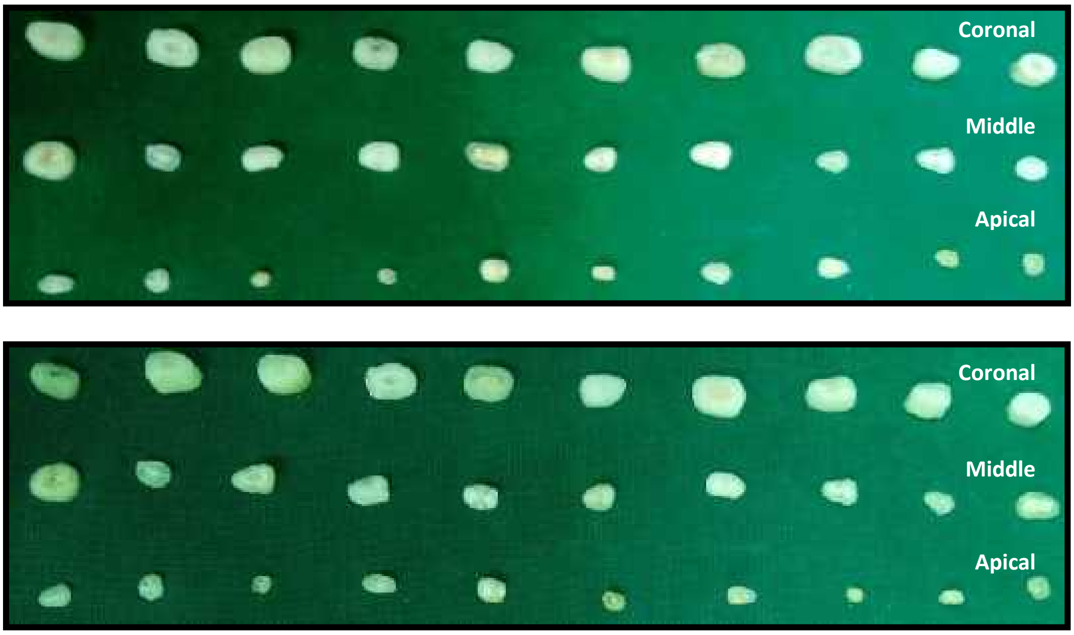


Fig 4.Transverse section of obturated samples for evaluation of sealer penetration in Group B(1% Phytic Acid)

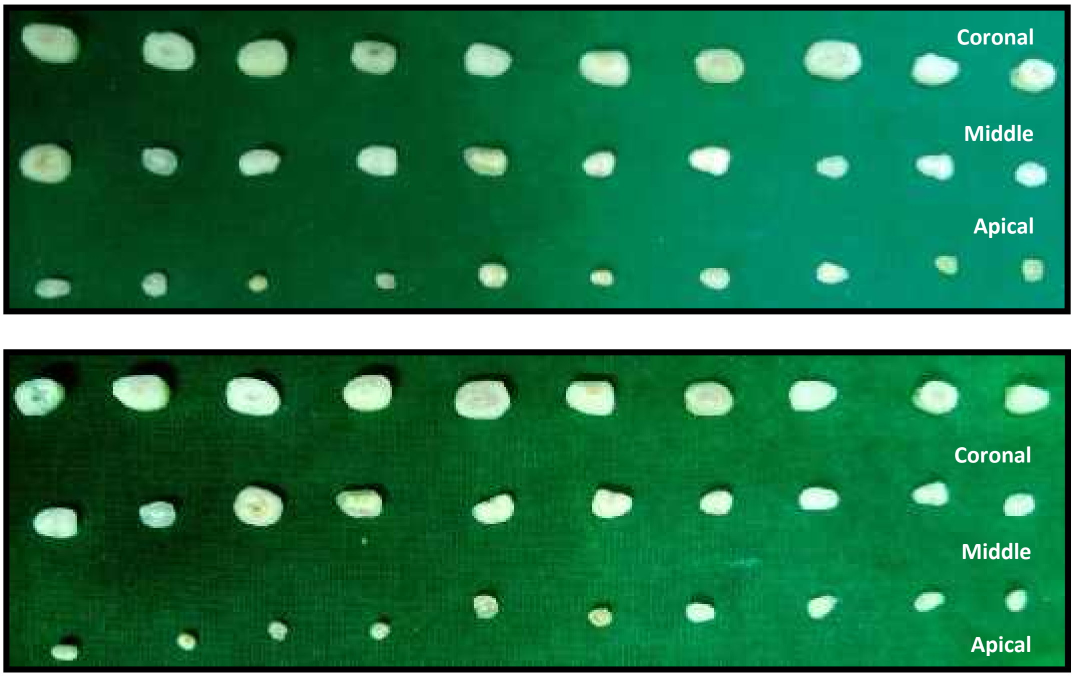


Fig 5. Transverse section of obturated samples for evaluation of sealer penetration in Group C(distilled water)

Fig 6. AH Plus sealer.

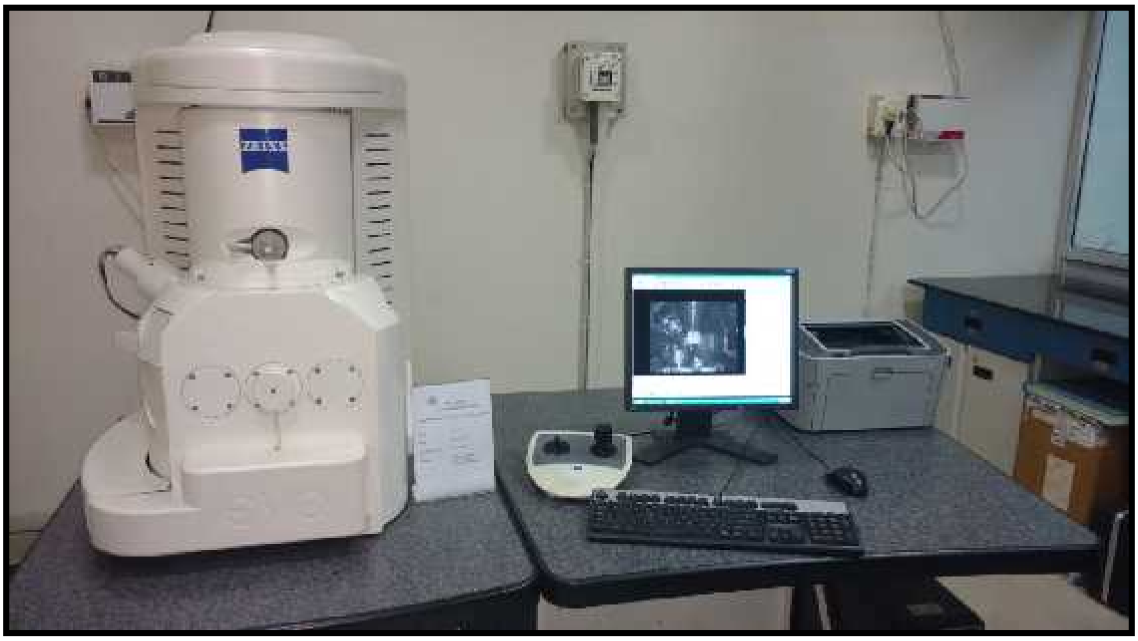


Fig 7. Scanning Electron Microscope

Fig 8. Sputter coating unit

Figure 9: Armamentarium For Root Canal Preparation

Fig 10. 17% EDTA solution

Fig 11. 5.25% Sodium hypochlorite solution

Fig 12. Phytic acid

Fig 13. 1% phytic acid

Fig 14. Dehydrator

RESULTS

The present in-vitro study was to evaluate and compare the penetration of AH Plus sealer into dentinal tubules by using scanning electron microscope following treatment with two different chelating agents. Sealer penetration was estimated using scanning electron microscopy images by calculating the distance from the sealer-gutta-percha interface to the root dentin in micrometers from each samples with n=10 with magnification range. (1500X – 2000X).

Mean and standard deviations were estimated from the samples with (n=20) for each study group. The results of the present study was subjected to statistical analysis to interpret the significant differences in assessing the penetrability and shear bond strength. One-way ANOVA followed by Tukey’s test was used for statistical analysis in the present study.

One-way analysis of variance (ANOVA) was used to study the overall variance within groups. It was not possible to identify the difference within the groups with the help of the P values obtained from ANOVA. Therefore a specific statistical test was used for intra-group comparison.

Tukey’s post hoc test was employed to do multiple comparison in between the group and within groups. All statistical analysis were done at the 0.05 significant level. SPSS version 19.0 was used to perform all statistical analysis.

In the present study, One-way ANOVA test showed a statistically significant difference among various groups due to the differences in mean penetration of the sealers used with P value of 0.000 which denotes significant level at 1%.From all the 3 groups, dentinal sections were evaluated at the 3, 5 and 8 mm levels. The mean and standard deviations of sealer penetration depths are presented.

Summary of the results:

Mean sealer penetration was found to be high in group A(EDTA) followed by group B(1% Phytic acid) least with group C(distilled water). Statistically significant difference was found in depth of sealer penetration in group A when compared with other groups. Though the penetration depth found in group B was lesser than group A they were statistically significant.

In all the groups mean sealer penetration was found to be high in cervical third followed by middle third and least in apical third and the difference was also statistically significant.

Overall ranking of sealer penetration evaluated in the study:

Group A>group B>group C

Table 01. Sealer penetration values in micrometers for Group A(EDTA)

S.no	Coronal	Middle	Apical
01	525.6	135.4	75.2
02	496.3	129.6	64.4
03	510.6	134.3	73.6
04	396.4	144.3	66.7
05	540.3	139.7	86.8
06	296.4	140.3	80.3
07	340.2	162.8	76.3
08	326.4	126.3	33.9
09	317.3	140.6	48.6
10	412.6	139.6	37.3
11	396.2	142.3	44.6
12	344.6	122.8	54.8
13	297.6	156.4	39.6
14	342.4	145.2	62.8
15	297.3	139.3	66.3
16	256.4	144.8	74.8
17	322.6	138.4	66.3
18	366.3	139.7	68.3
19	396.4	144.3	74.6
20	320.6	145.6	68.3

Table 02. Sealer penetration values in micrometers for Group B(1% Phytic Acid)

S no	Coronal	Middle	Apical
01	249.3	126.3	68.6
02	286.3	120.2	39.4
030	245.6	75.6	30.6
04	239.8	94.2	44.8
05	190.6	98.6	66.9
06	222.4	77.4	72.4
07	286.4	65.2	68.3
08	300.8	101.4	44.6
09	148.6	111.3	32.8
10	166.8	93.6	29.6
11	175.6	88.7	40.2
12	188.4	64.3	26.8
13	145.3	114.3	32.9
14	166.4	126.3	60.6
15	182.6	145.8	52.3
16	212.9	140.3	56.8
17	202.4	112.8	69.2
18	112.6	104.6	40.3
19	124.2	118.3	22.8
20	130.6	144.2	29.3

Table 03. Sealer penetration values in micrometers for Group C(distilled water)

S no	Coronal	Middle	Apical
01	110.2	58.4	46.4
02	94.1	54.6	33
03	80.2	39.2	25
04	76.4	36.6	22
05	64.2	38.6	31.2
06	78	44.2	27
07	92.5	57.3	37.6
08	104.1	62.5	29.8
09	96.4	38.3	26.8
10	99.2	61	32.6
11	102.1	54.3	27
12	113.6	51	22.2
13	96.2	59	29.2
14	91.2	36.2	20.5
15	86.3	49	30.3
16	88.3	44.4	25.3
17	93.4	38.6	21.2
18	79.2	66	35.5
19	66.1	46	32.3
20	68.1	37	25.3

Table 04. One-way with mean and standard deviation for sealer penetration for different levels

Oneway

Descriptives

Levels		N	Mean	Std. Deviation	Std.Error	95% confidence interval for mean		Minimum	Maximum
						Lower bound	Upper bound		
CORONAL	EDTA	20	375.1250	83.61135	18.69607	335.9937	414.2563	256.40	540.30
	Phytic Acid	20	198.8800	55.72634	12.46079	172.7993	224.9607	112.60	300.80
	Distilled water	20	88.9900	14.07611	3.14751	82.4022	95.5778	64.20	113.60
	Total	60	220.9983	132.06335	17.04931	186.8828	255.1139	64.20	540.30
MIDDLE	EDTA	20	140.5850	9.04621	2.02279	136.3512	144.8188	122.80	162.80
	Phytic Acid	20	106.1700	24.44687	5.46649	94.7285	170.6150	64.30	145.80
	Distilled water	20	48.6100	9.93282	2.22105	43.9613	53.2587	36.20	66.00
	Total	60	98.4550	41.40834	5.34579	87.7581	109.1519	36.20	162.80
APICAL	EDTA	20	63.1750	15.11130	3.37899	56.1027	70.2473	33.90	86.80
	Phytic Acid	20	44.9600	15.70576	3.51192	37.6095	52.3105	22.80	72.40
	Distilled water	20	29.0100	6.28523	1.40542	26.0584	31.9516	20.50	46.40
	Total	60	45.7150	19.07415	2.46246	40.7876	50.6424	20.50	86.80

Table 05. ANOVA for sealer penetration for different levels

ANOVA

		Sum of squares	df	Mean square	F	Sig.
CORONAL	Between groups	833409.002	2	416704.501	121.436	0.000
	Within groups	195593.968	57	3431.473		
	Total	1029002.970	59			
MIDDLE	Between groups	86379.643	2	43189.821	166.511	0.000
	Within groups	14784.746	57	259.832		
	Total	101164.388	59			
APICAL	Between groups	11689.573	2	5844.786	34.079	.000
	Within groups	9776.003	57	171.509		
	Total	21465.576	59			

Table 06. Multiple comparison for sealer penetration for different levels (Tukey's test)

Post Hoc Tests

Tukey HSD

Multiple Comparisons

Dependent variable	chelating agent(I)	chelating agent(J)	Mean difference(I-J)	Std.Error	Sig.	95% Confidence Interval	
						Lower bound	Upper bound
CORONAL	EDTA	PHYTIC ACID	176.24500*	18.52424	.000	131.6679	220.8221
		DISTILLED WATER	286.13500*	18.52424	.000	241.5579	330.7121
	PHYTIC ACID	EDTA	-176.24500*	18.52424	.000	-220.8221	-131.6679
		DISTILLED WATER	109.89000*	18.52424	.000	65.3129	154.4671
	DISTILLED WATER	EDTA	- 286.13500*	18.52424	.000	-330.7121	-241.5579
		PHYTIC ACID	- 109.89000*	18.52424	.000	-154.4671	-65.3129
MIDDLE	EDTA	PHYTIC ACID	34.41500*	5.09295	.000	22.1592	46.6708
		DISTILLED WATER	91.97500*	5.09295	.000	79.7192	104.2308
	PHYTIC ACID	EDTA	-34.41500*	5.09295	.000	-46.6708	-22.1592
		DISTILLED WATER	57.56000*	5.09295	.000	45.3042	69.8158
	DISTILLED WATER	EDTA	-91.97500*	5.09295	.000	-104.2308	-79.7192
		PHYTIC ACID	-57.56000*	5.09295	.000	-69.8158	-45.3042
APICAL	EDTA	PHYTIC ACID	18.21500*	4.14136	.000	8.2492	28.1808
		DISTILLED WATER	34.16500*	4.14136	.000	24.1992	44.1308
	PHYTIC ACID	EDTA	-18.21500*	4.14136	.000	-28.1808	-8.2492
		DISTILLED WATER	15.95000*	4.14136	.001	5.9842	25.9158
	DISTILLED WATER	EDTA	-34.16500*	4.14136	.000	-44.1308	-24.1992
		PHYTIC ACID	-15.95000*	4.14136	.001	-25.9258	-5.9842

* The mean difference is significant at the 0.05 level.

Table 07. One-way with mean and standard deviation for sealer penetration for different Groups.

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	maximum
						Lower bound	Upper bound		
EDTA	CORONAL	20	375.1250	83.61135	18.69607	335.9937	414.2563	256.40	540.30
	MIDDLE	20	140.5850	9.04621	2.02279	136.3512	144.8188	122.80	162.80
	APICAL	20	63.1750	15.11130	3.37899	56.1027	70.2473	33.90	86.80
	TOTAL	60	192.9517	142.26660	18.36654	156.2103	229.732	33.90	540.30
PHYTIC ACID	CORONAL	20	198.8800	55.72634	12.46079	172.7993	224.9607	112.60	300.80
	MIDDLE	20	106.1700	24.44687	5.46649	94.7285	117.6115	64.30	145.80
	APICAL	20	44.9600	15.70576	3.51192	37.6095	52.3105	22.80	72.40
	TOTAL	60	116.6700	73.09923	9.43737	97.7865	136.5535	22.80	300.80
DISTILLED WATER	CORONAL	20	88.9900	3.14751	3.14751	82.4022	95.5778	64.20	113.60
	MIDDLE	20	48.6100	2.22105	2.22105	43.9613	53.2587	36.20	66.00
	APICAL	20	29.0100	1.40542	1.40542	26.0684	31.9516	20.50	46.40
	TOTAL	60	55.5367	27.24811	3.51772	48.4977	62.5758	20.50	113.60

Table 08. ANOVA for sealer penetration for different Groups.

		Sum of squares	df	Mean Square	F	Sig.
EDTA	Between Groups	1055427.481	2	527713.741	216.838	.000
	Within Groups	138719.820	57	2433.681		
	Total	1194147.302	59			
PHYTIC ACID	Between Groups	240221.164	2	120110.582	91.229	.000
	Within Groups	75045.162	57	1316.582		
	Total	315266.302	59			
DISTILLED WATER	Between Groups	37415.365	2	18707.683	166.883	.000
	Within Groups	6389.734	57	112.101		
	Total	43805.099	59			

Table 09. POST HOC TESTS

Multiple comparison for sealer penetration for different Groups.

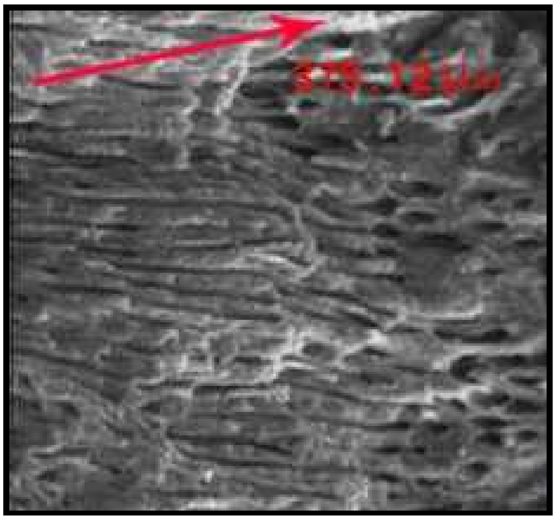
Tukey HSD

Multiple Comparison

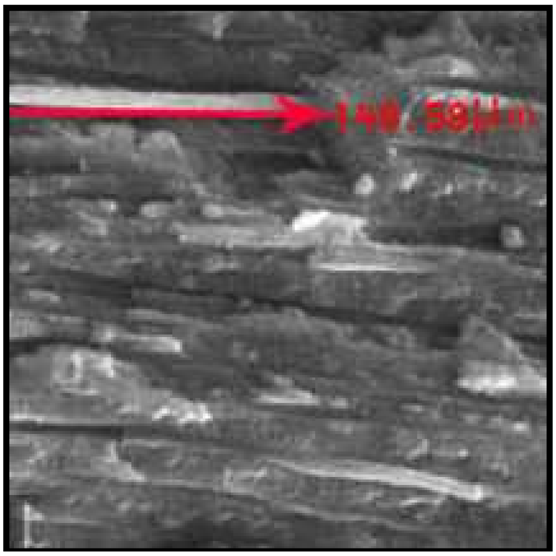
Dependent variable	(I)Portion of Root	(J)Portion of Root	Mean Difference(I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
EDTA	CORONAL	MIDDLE	234.54000*	15.60026	.000	196.9993	272.0807
		APICAL	311.95000*	15.60026	.000	274.4093	349.4907
	MIDDLE	CORONAL	-234.54000*	15.60026	.000	-272.0807	-196.9993
		APICAL	77.41000*	15.60026	.000	39.8693	114.9507
	APICAL	CORONAL	-311.95000*	15.60026	.000	-349.4907	-274.4093
		MIDDLE	-77.41000 *	15.60026	.000	-114.9507	-39.8693
PHYTIC ACID	CORONAL	MIDDLE	92.71000*	11.47424	.000	65.0982	120.3218
		APICAL	61.21000*	11.47424	.000	126.3082	181.5318
	MIDDLE	CORONAL	-92.71000*	11.47424	.000	-120.3218	-65.0982
		APICAL	61.21000*	11.47424	.000	33.5982	88.8218
	APICAL	CORONAL	-153.92000*	11.47424	.000	-181.5318	-126.3082
		MIDDLE	-61.21000*	11.47424	.000	-88.8218	-33.5982
DISTILLED WATER	CORONAL	MIDDLE	40.38000*	3.34814	.000	32.3230	48.4370
		APICAL	59.98000*	3.34814	.000	51.9230	68.0370
	MIDDLE	CORONAL	-40.38000*	3.34814	.000	-48.4370	- 32.3230
		APICAL	19.60000*	3.34814	.000	11.5430	27.6570
	APICAL	CORONAL	-59.98000*	3.34814	.000	-68.0370	-51.9230
		MIDDLE	-19.60000*	3.34814	.000	-27.6570	- 11.5430

* The mean difference is significant at the 0.05 level.

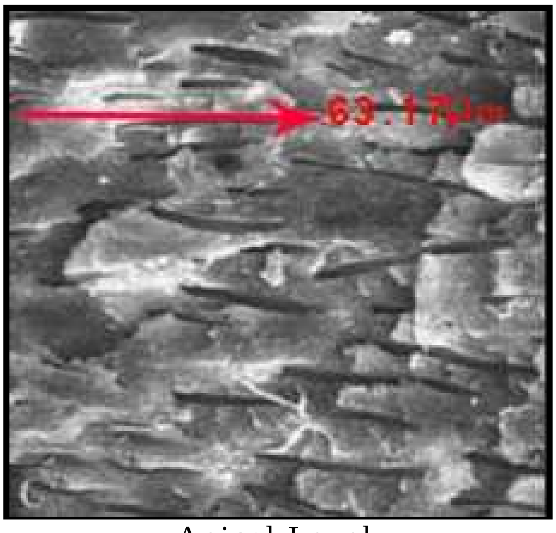
Group A (17% EDTA)



Coronal Level

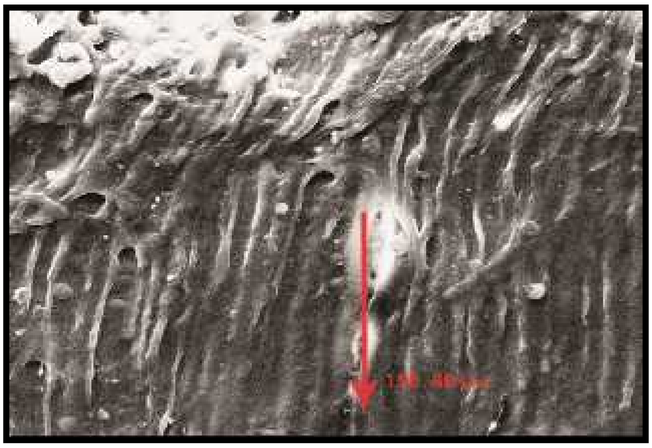


Middle Level

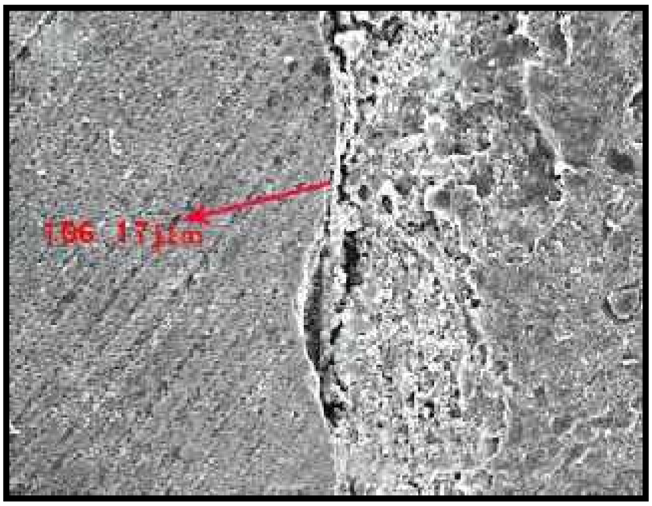


Apical Level

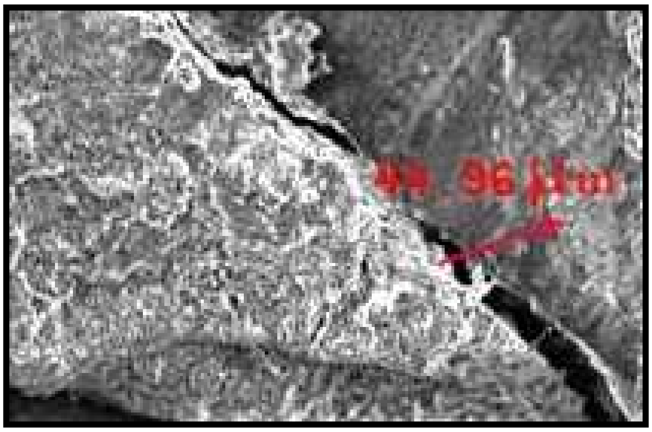
Group B (1% Phytic Acid)



Coronal Level

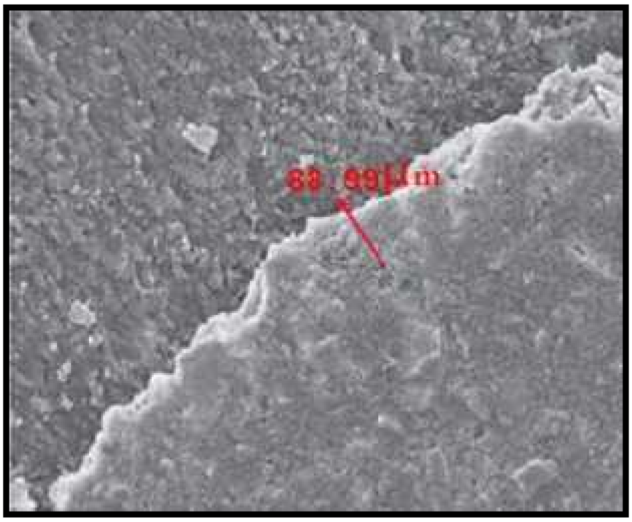


Middle Level

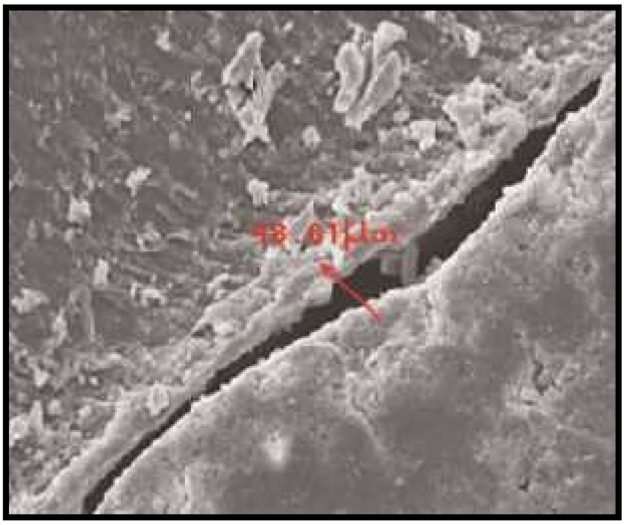


Apical Level

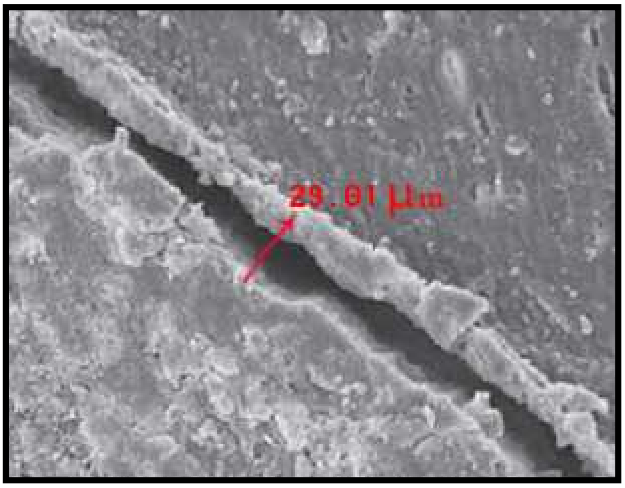
Group C (Distilled Water)



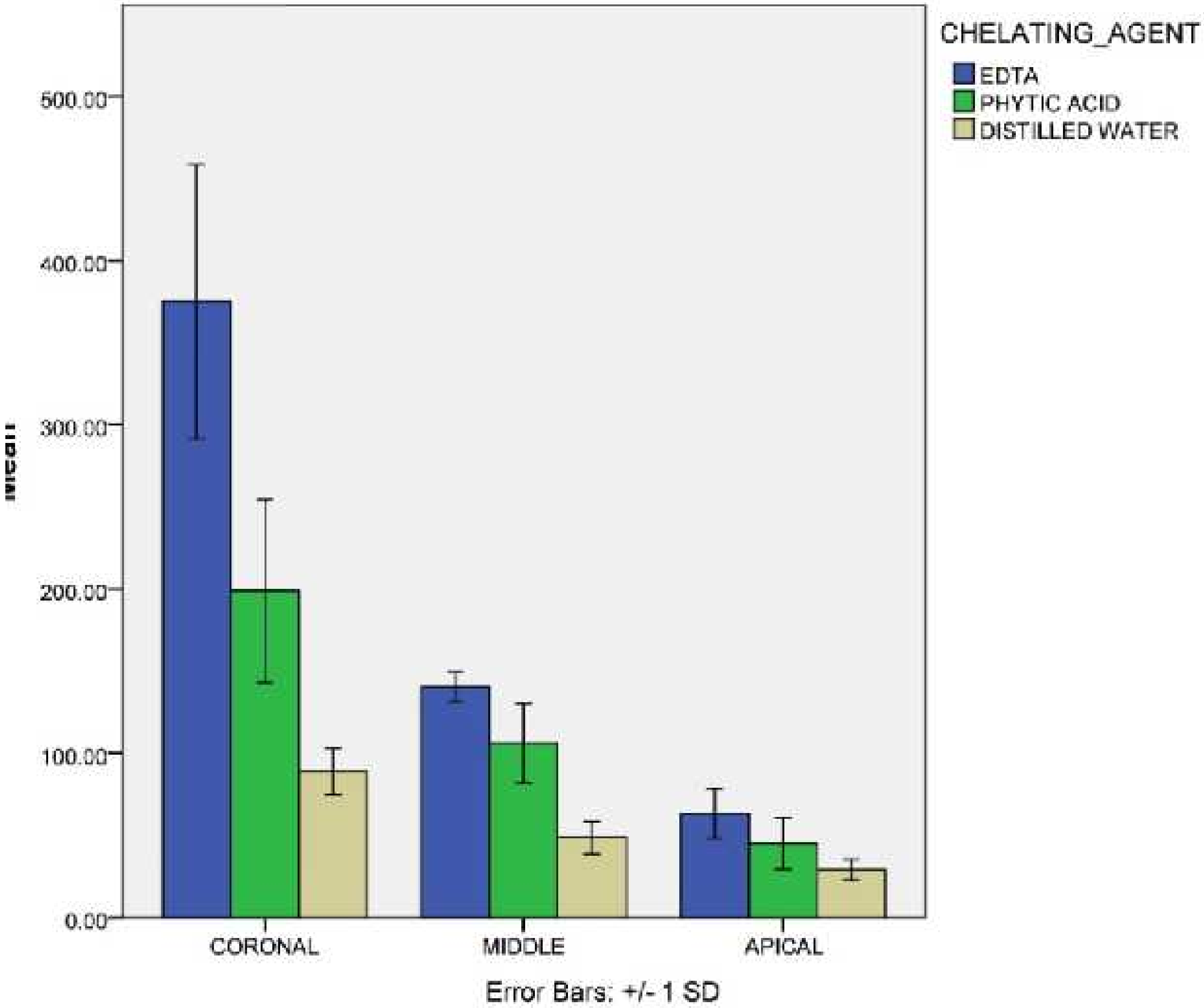
Coronal Level



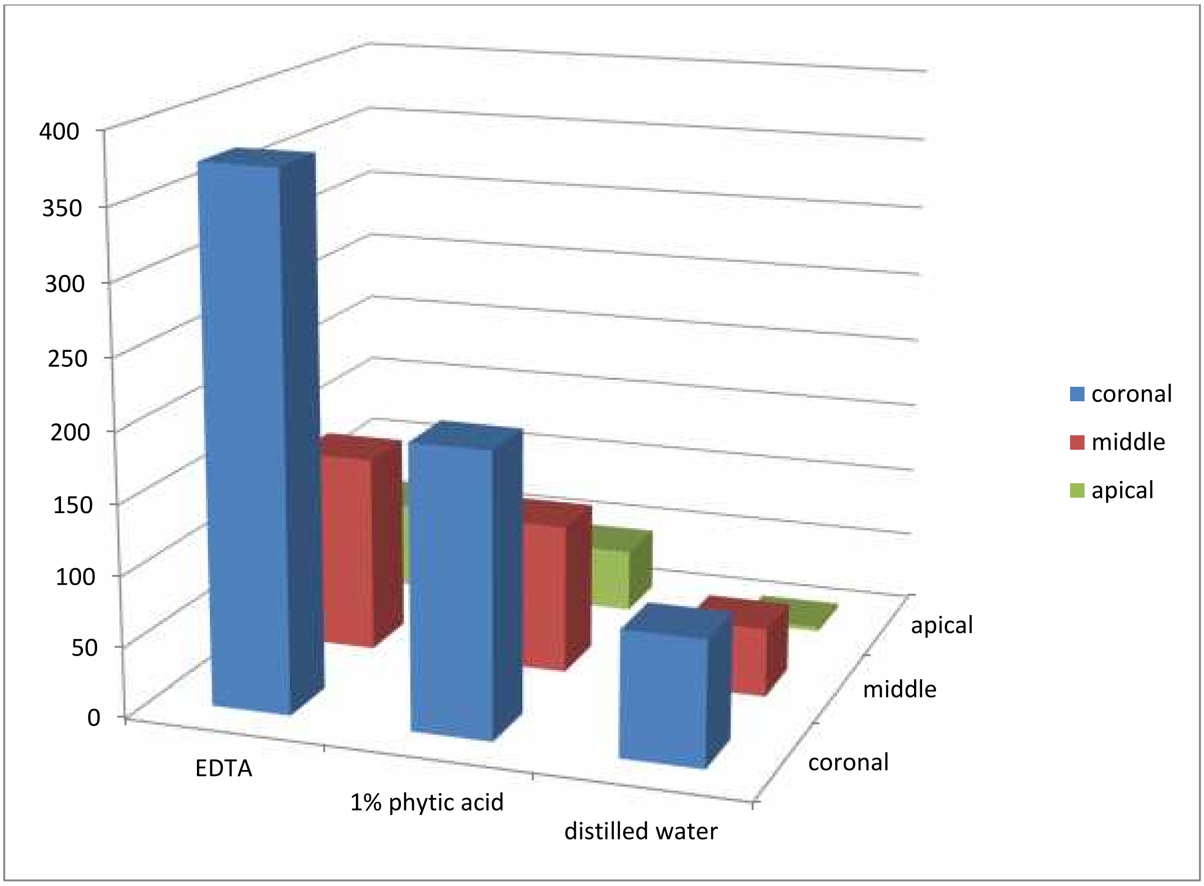
Middle Level



Apical Level



Graph 1. Mean sealer penetration values for tested chelating agents (Bar Diagram)



Graph 2. Mean sealer penetration values for coronal, middle and apical thirds for tested chelating agents.

DISCUSSION

The basic requirements of root canal treatment are effective chemo mechanical preparation and three dimensional obturation of the root canal system. Chemomechanical preparation involves debulking of bacterial load and debris in root canal space. The success in achieving of through debridement involves complete removal of bacterial pathogens located in apical third. The chemicals used during the above process aids in achieving the above goal by sterilizing the endodontic space and improve quality of periapical tissues.

Subsequent to sufficient chemomechanical preparation, a hermetic sealing with a biocompatible material is another important objective of root canal treatment. According to Grossman,[84] an ideal endodontic sealer should have good adaptation to the root dentin and core filling material, good rheological behavior, adequate lubricant action, last solubility, high antibacterial activity, should be easy to manipulate and should possess adequate dimensional stability.

AH Plus sealer shows best results for adaptation to the root canal wall, tubular penetration and adaptation to the peritubular dentin directly. These findings are supported by other studies.[33,34]

The good penetration, adaptation and adhesion properties will have 2 positive effects,[61] in first place on sealing because of the increased, surface contact between sealer and dentin and second on the antimicrobial effect by locking the residual microorganism in the dentinal tubules. Hence, we have used AH Plus sealer to evaluate sealer penetration depth in our study.

The sealer penetration depth in the dentinal tubules depends on many factors like smear layer removal,[85] dentinal permeability, root canal dimension, and the physical and chemical properties of the sealer.[86,87,88]

According to Boyde,[89] the smear layer is an organic matter trapped within translocated inorganic dentine and is formed during instrumentation which is composed of organic and inorganic substances that include fragments of odontoblastic processes, microorganisms and necrotic materials. Smear layer plays a major role in the penetration of root canal sealers. Removal of smear layer reduces the number of intracanal microorganisms, enhances the sealing properties of root canal filling materials, increases bond strength to dentinal walls and is removed using various demineralizing agents.

Since the smear layer contains both organic and inorganic components, its removal usually requires a combination of NaOCl(organic solvent) and acids such as citric, tannic, polyacrylic or chelating agents such as EDTA.

Goldman et al[38] examined the effect of various combination of EDTA and NaOCl as irrigation solution and after instrumentation. The most effective irrigation solution was 5, 25 % NaOCl and the most effective final flush was 10 ml of 17% EDTA followed by 10 ml of 5.25% NaOCl, which was also confirmed by Yamada et al.[39] The time of exposure also affects the performance. Root canal dentine showed reverse peritubular and intratubular erosions after 10 minutes irrigation with liquid EDTA(17%), whereas 1 minute exposure was effective in removing the smear layer. This finding was also supported by studied from Yamada et al and Sudha et al.[90]

Hence, in our present study we used chelating agent EDTA at a concentration of 17% and exposure time was limited to one minute to have effective smear layer removal without affecting peritubular and intertubular tissues.

Nygaard- Ostby[11] investigated the effect of 15% EDTA solution on human periapical tissue as well as pulpal tissue and found no periapical tissue damage defected after a period of action of upto 14 months. In contrast, Collet et al[91] reported that 15% sodium (Na)-EDTA showed toxic effects in vitro complete prevention of cell growth was detected after in vitro use of EDTA-T Segura et al[92] also showed that extrusion of even low concentration of EDTA solutions through the apical constriction not only resulted in an irreversible decalcification of periapical bone, but also influenced neuroimmunological regulatory mechanisms.

Though EDTA has been the most commonly used irrigant since 1957,[11] the extrusion of EDTA beyond root canal is a cause of concern due to its effects on periapical, pulpal tissues. Also it is commonly synthesized on an industrial scale from which results formation of impurities which are major organic pollutants. Considering these facts is warranted, and the search for more biocompatible material to replace EDTA is still going on.

Phytic acid(IP6, inositol hexakisphosphate) is the major storage form of phosphorus in plant seeds and rice bran that contributes in a variety of cellular functions.[21] It is also omnipresent in mammalian cells, with a concentration ranging from 10to 100 µmol/L. IP6 can be extracted with low cost from rice bran. This agent has multiple negative charges, making it an effective chelator of multivalent cations such as calcium(Ca), magnesium and iron.[23,24] On the basis of these properties, IP6 was evaluated as a root canal chelating agent as an alternative to EDTA. Nasser et al[75] in their study found that IP6 was effective in removing the smear layer from NaOCl treated flat coronal dentin surfaces and instrumented root canals. IP6 is highly negatively charged molecule that has affinity to Ca^{2+}. Flat coronal dentin surfaces treated 9% IP6 were cleaned with more widely open dentinal tubules when compared with EDTA. On root canal surfaces, the effect of both IP6 and EDTA in cleaning the apical third was less than that of in middle third, and this is attributed to the anatomy of the former region. Both acidity and chelating function of IP6, makes it an effective smear layer removal agent.

They also studied the chelating ability of 1% phytic acid found that 1% phytic acid has good chelating effect. They also found that 17% EDTA caused significant suppression of ALP activity whereas presence of IP6 in the culture medium did not affect the viability, morphology, or ALP activity of the cells. IP6 was reported to have a double side in cell culture as an iron chelator and a source of phosphate for cells. IP6 protects the cells from oxidation injury through binding to ion, a metal that catalyses the formation of hydroxyl radicals. Hence they are proved to be bio compatible.

Nasser et al in 2013[69] in their study found similar results. IP6 proved to be an effective agent for removing the smear layer, and did not have negative effect on pulpal cells. In addition, IP6 etched dentin showed a increased resin-dentin bond strength. In addition to completely removing the smear layer as well as slightly etching the dentin, IP6 was effective in preventing collagen degradation of bacteria collagenase.

In study by Saketh et al[83] Phytic acid alone showed more zone of inhibition used alone. In this study, MIC tests showed that effective

IP6 concentration was lower than those of phytic acid,0.156% vs 0.578% respectively. The MIC value of IP6 was close to MIC value of EDTA AND NaOCl 0.14% and 0.093% respectively. IP6 showed bactericidal activity against E. faecalis at 0.625%.

IP6 has good chelating effect as shown by complete smear layer removal effect, has slight etching effect on dentin, has antimicrobial effect, is biocompatible, prevents collagen degradation of bacteria collagenase. Hence we evaluated the chelating effect of IP6 for penetration effect of sealer into dentinal tubules.

The study of interface sealer/dentin by scanning electron microscopy can be done with longitudinal or cross-sectional sections. The direction of the tubules is mostly perpendicular to the root canal wall. For both cutting directions, probability to obtain a section longitudinal of tubule is equal. Longitudinal sections are used in most studies. They are appropriate when coronal or middle part of the root canal is evaluated or when location of the evaluation is not mentioned.[93,94] However, for thin or curved roots, this could create problems in the apical root canal; therefore we used cross-sectional sections.

In this study scanning electron microscope was utilized to estimate the mean penetration of root canal sealers. Scanning Electron Microscopic micrographs allow for observation of the dentinal tubules and accurate measurement of penetration depth of the sealers into dentinal tubules at a high magnification. In addition it allows for the observation of sealer within dentinal tubules at distant sites from the root canal where the density of the tubules is lower. Main disadvantage of this technique is inability to obtain detailed view at low magnification and artifacts during specimen preparation may affect results.

In the coronal third of root canal mean penetration values were:

- Group A(375.12µm) > Group B(198.88µm) > Group C(88.99µm)
- In the middle third of root canal mean penetration values were
- Group A(140.58µm) > Group B(106.17µm) > Group C(48.61µm)
- In the apical third of the root canal mean penetration values were
- Group A(63.17µM) > Group B(44.96µm) > Group C(29.01µm)

In present study EDTA group had highest mean penetration values in all the levels of root canal. When compared to Phytic acid group and Distilled water the penetration values was highly significant indicating EDTA to be a better chelating agent than other two variables.

This significant difference was observed in all the levels of root canals also.

When penetration of sealer in EDTA group was evaluated, highest mean penetration values was seen in coronal region(375.1µm) followed by middle region(140.5µm), least penetration values were seen at apical region(63.17µm).

Similar results were seen in studies by Bulguerrie at al, Weis MV et al, Sevimay S et al where penetration in dentinal tubules was significantly greater in coronal and middle part of the root canal than the apical root.

Phytic acid group had mean penetration values lesser than that of EDTA group but higher than that of Distilled water.

Though the values are less than that of EDTA, their difference was statistically significant. This result was observed in all these levels of root canals.

When penetration of sealer in Phytic acid was evaluated, highest mean penetration values was seen in coronal region(198.88)µm, followed by middle region(106.17µm), least for apical region(44.96µm).

Distilled water group had the least penetration values at all levels of root canals even in this group it was observed that coronal region had better penetration value followed by middle and least penetration values were seen in apical region.

Better penetration values in coronal and middle thirds may be a result of the removal efficiency of smear layer in coronal and middle thirds of the canals. Also it is known that the number of dentinal tubules and the size of their lumens are far greater in the coronal than in the apical area, they allow for better adhesion of the sealer to the root canal walls(Vassilidas et al 1996).[95]

Least penetration was seen in apical region in all three groups. This could be because apical root canal contains less tubules, and when present, the diameter is smaller or they are more often closed. The apical portion of roots shows a large variation in structure. Dentinal tubules are irregular in direction and density, some areas are devoid of tubules. Sometimes, cementum like tissue can line the apical root canal wall, occluding any tubules.

SUMMARY AND CONCLUSION

The success of root canal treatment depends on cleaning and shaping, followed by three-dimensional obturation of the root canal system to prevent reinfectiion. Tubular penetration and adaptation of sealer can determine the sealability of root filling which in turn are determined by many factors like smear layer removal, dentinal permeability, root canal dimension, and the physical and chemical properties of the sealer. Smear layer removal forms an important determining factor in sealer penetrability. Traditionally, it is done with 5.25% NaOCl irrigation followed by 17 % EDTA. Since EDTA is not biodegradable and its possible damage to periapical tissues on extrusion, search for an alternative chelating agent is going on.

Phytic acid, known as inositol hexakisphosphate(IP6), is a naturally occurring agent, has ability to chelate with positively charged multivalent cations while having minimal effect on pulpal cells, we evaluated 1% phytic acid for its chelating ability.

The aim of the present in-vitro study was to evaluate and compare the penetration of AH Plus sealer into dentinal tubules by using scanning electron microscope following treatment with two different chelating agent i.e 17% EDTA and 1% phytic acid.

Sixty freshly extracted human mandibular first premolar with single straight root canals were used in the study. These were randomly divided into three equal groups of 20 samples each. The crowns of all teeth were cut at Cemento-enamel junction using high speed tapering diamond under air water spray with remaining root length 12±1mm. The working length were established by placing a size 10 K file into each sample until the tip of the file was visible at the apex. Canal length was established 1 mm short of the apex. The root canals were prepared using the ProTaper rotary system to an apical size of F3, and apical patency was rechecked using size-10 K- file throughout the preparation. During the entire preparation, alternate irrigation and recapitulation was done with 5.25% sodium hypochlorite (NaOCl) (Avarice Laboratory, Ghaziabad, India) and #10 K-file, respectively.

Samples were divided into 3 groups(A,B,C) with20 samples each.

1. Group A (EDTA): samples(n=20) were irrigated with 10ml of 17% EDTA for 1min.
2. Group B (1% Phytic acid): samples(n=20) were irrrigated with 10ml of 1%phytic acid for 1min.
3. Group C (distilled water): samples(n=20) were irrigated with 10ml distilled water for 1min.

After irrigation with different irrigating agents all root canals were obturated with help of GP and AH-Plus sealer by lateral condensation techniques. Samples were then sectioned in the bucco-lingual direction with the help of sorensen disc Smear layer produced during sectioning were removed by cleansing with 17% EDTA and 3% Naocl. Samples were studied for dentinal tubule penetration at all the three levels - coronal, middle and apical levels. The penetration of sealer into the dentinal tubules were assessed by using scanning electron microscopic(SEM) examination.

It was found that highest sealer penetration depth was found in group A (EDTA) followed by group B least with group C. Though the penetration in group B was lower than group A it had significant levels of penetration indicating a potential chelating effect. In all three groups coronal region had highest levels of penetration followed by middle region and least in apical regions.

Within the limitations of the study it can be concluded that EDTA group had highest sealer penetration. Phytic acid group had intermediate effect indicating potential chelating ability. Further studies are essential to confirm required concentration, pH, exposure time for its optimal chelating effect for using it as an alternative chelating agent.

REFERENCES

1. Violich D, Chandler N. The smear layer in endodontics–a review. Int Endod J. 2010;43(1):2-15.
2. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod. 1975;1(7):238-42.
3. Wauters T, Wauters J. Should the dentin smear layer be preserved or eliminated?. Rev Belge Med Dent (1984). 1999;55(2):93-103.
4. Zand V, Bidar M, Ghaziani P, Rahimi S, Shahi S. A comparative SEM investigation of the smear layer following preparation of root canals using nickel titanium rotary and hand instruments. J Oral Sci. 2007;49(1):47-52.
5. Lui J-N, Kuah H-G, Chen N-N. Effect of EDTA with and without surfactants or ultrasonics on removal of smear layer. J Endod. 2007;33(4):472-5.
6. Dadresanfar B, Darmiani S, Moshari A. Effect of Master Apical File Size and Taper on Irrigation and Cleaning of the Apical Third of Curved Canals. J Dent (Tehran). 2014;11(2):188-95.
7. Shahravan A, Haghdoost A-A, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: a systematic review and meta-analysis. J Endod. 2007;33(2): 96-105.

8. Callahan JR. Sulphuric acid for opening root canal. Dental Cosmos 1894;36:957-959.
9. Nikiforuk G, Sreenby L. Demineralisation of hard tissues by organic chelating agents at neutral pH. J Dent Res 1953;32:859-867.
10. Hahn F, Reygadas F. Demineralisation of hard tissues. Science 1951;114:462-463.
11. Nygaard-Ostby B. Chelation in root canal therapy:ethylene-ediaminetetraacetic acid fir cleansing and widening of root canals. Odont Tids 1957;65:3-11.
12. Von der Fehr F, Nygaard-Ostby B. Effect of EDTAC and sulphuric acid on root canal dentine. Oral Surg Oral MednOral Pathol 1963;16:199-205.
13. Stewart G, kapsimalis P, Rappaport H. EDTA and urea peroxide for root canal preparation. J Am Dent Assoc 1969;78:335-358.
14. Kaufman, AY, Binaderman I, Tal M Gedalia I, Peretz O. New chemotherapeutic agent for root canal treatment. Oral Surg Oral Med Oral Pathol 1978;48:283-295.
15. Scleza M, Antoniazzi J, Scleza P. Efficacy of final irrigantion – a scanning microscopic evaluation. J Endod 2000;26:355-358.
16. Calt S, Serper A. Time dependent effects of EDTA on dentine structures. J Endod 2002;28:17–9.
17. Elvers B. Ullmann’s Fine Chemicals. Weinheim: Weinheim, Germany: Wiley-VCH;ww 2014:647.

18. Sillanpaa M. Environmental fate of EDTA and DTPA. Rev Environ Contam Toxicol 1997;152:85–111.

19. Segura JJ, Calvo JR, Guerrero JM, et al. The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: endodontic implications. J Endod 1996;22:337–40.

20. Amaral KF, Rogero MM, Fock RA, et al. Cytotoxicity analysis of EDTA and citric acid applied on murine resident macrophages culture. Int Endod J 2007;40:338–43.

21. Kuwano M, Mimura T, Takaiwa F, Yoshida KT. Generation of stable 'low phytic acid' transgenic rice through antisense repression of the 1D-myo-inositol 3-phosphate synthase gene (RINO1) using the 18-kDa oleosin promoter. Plant Biotechnol J 2009; 7: 96–105.

22. Hurrell RF. Influence of vegetable protein sources on trace element and mineral bioavailability. J Nutr 2003; 133: 2973S–2977S.

23. Schlemmer U, Frolich W, Prieto RM, Grases F. Phytate in foods and significance for humans: food sources, intake, processing, bioavailability, protective role and analysis. Mol Nutr Food Res 2009; 53(Suppl 2): 330S–375S.

24. Lopez-Gonzalez AA, Grases F, Monroy N, Mari B, Vicente-Herrero MT, Tur F, Perello J. Protective effect of myoinositol hexaphosphate (phytate) on bone mass loss in postmenopausal women. Eur J Nutr 2013; 52: 717–726.

25. Ravichandran R, Seitz V, Reddy Venugopal J, Sridhar R, Sundarrajan S, Mukherjee S, Wintermantel E, Ramakrishna S. Mimicking native extracellular matrix with phytic acid-crosslinked protein nanofibers for cardiac tissue engineering. Macromol Biosci 2013; 13: 366–375.
26. Borggreven JM, Driessens FC. Effect of phytate and hexadecylamine on the permeability of bovine dental enamel. Arch Oral Biol 1983; 28: 375–379.
27. Nordbo H, Rolla G. Desorption of salivary proteins from hydroxyapatite by phytic acid and glycerophosphate and the plaque-inhibiting effect of the two compounds in vivo. J Dent Res 1972; 51: 800–811.
28. Prosser HJ, Brant PJ, Scott RP, Wilson AD. The cement forming properties of phytic acid. J Dent Res 1983; 62: 598–600.
29. Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297–306.
30. Evans JT, Simon JHS. Evaluation of the apical seal produced by injected thermoplasticized gutta-percha in the absence of smear layer and root canal sealer. J Endod 1986;12:101–7.
31. Wu MK, De Gee AJ, Wesselink PR. Effect of tubule orientation in the cavity wall on the seal of dental filling materials: an in vitro study. Int Endod J 1998;31:326–32.

32. Heling I, Chandler NP. The antimicrobial effect within dentinal tubules of four canal sealers. J Endod 1996;22:257–9.
33. Sen BH, Piskin B, Baran N. The effect of tubular penetration of root canal sealers on dye microleakage. Int Endod J 1996;29: 23–8.
34. Kokkas A, Boutsioukis A, Vassiliadis L, Stavrianos CK. Stereomicroscopique and scanning electron microscopic study of roots obturated with vertically condensed gutta-percha, epoxy resin cement, and dentin bonding agent. J Endod 2004;30:100–2.
35. Lee KW, Williams MC, Camps JJ, Pashley DH. Adhesion of endodontic sealers to dentin and gutta-percha. J Endod 2002;28:684–8.
36. Siqueira JF, Favieri A, Gahyva SMM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity of flow rate of newer and established root canal sealers. J Endod 2000;28:274–7.
37. Peters LB, Van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J 2002;35:13–21.
38. Goldberg F, Abramovich A. Analysis of the effect of EDTAC on the dentinal walls of the root canal. *Journal of Endodontics*. 1977; 3: 101-5.
39. Yamada RS, Armas A, Goldman M, Sun Lin P. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: part 3. J Endod 1983;9:137–42.

40. Berg MS, Jacobsen EL, BeGole EA, Remeikis NA. A comparison of five irrigating solutions: a scanning electron microscopic study. *Journal of Endodontics*. 1986; 12: 192-7.

41. Cergneux M, Ciucchi B, Dietschi JM, Holz J. The influence of the smear layer on the sealing ability of canal obturation. *International Endodontic Journal*. 1987; 20: 228-32.

42. Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. *Journal of Endodontics*. 1997; 23: 725-7.

43. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. *Journal of dentistry*. 1999; 27: 43-6.

44. Choudary M, Roopa M. Effect of EDTA, NaOCl and their combination at different time periods on smear layer: A SEM study. *JEndodontology*. 2000; 12: 71-6.

45. Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. *Journal of Endodontics*. 2002;28: 17-9.

46. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics: a review. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2002; 94: 658-66.

47. Schafer E, Zandbiglari T. Solubility of root –canal sealers in water and artificial saliva. *International Endodontic Journal*. 2003; 36: 660-669.

48. S.Sevimay & D.Dalat. evaluation of penetration and adaptation of three different sealers: a SEM study. Journal of Oral Rehabilitation 2003;30:951-955.

49. Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. *Journal of Endodontics*. 2004; 30: 792-5.

50. A.Khademi, M. Feizianfard. The effect of EDTA and citric acid on smear layer removal of mesial canals of first mandibular molars, A scanning electron microscopic study. J of Research in Medical Sciences 2004;2:80-88.

51. S. Sevimay & a. Kalayci, Evaluation of apical sealing ability and adaptation to dentine of two resin-based sealers, Journal of Oral Rehabilitation 2005 32; 105–110.

52. John I. Ingle, Carl W. Newton et al, obturation of the radicular space, chapter 11, BC Decker Inc, INGLE'S Text book of endodontics, CV Mosby, 5th edition, page.no: 572.

53. Dotto SR, Travassos RM, De Oliveira EP, de Lima Machado ME, Martins JL. Evaluation of ethylenediaminetetraacetic acid (EDTA) solution and gel for smear layer removal. *Australian Endodontic Journal*. 2007; 33: 62-5.

54. Christian Ralf Gernhardt, Thomas Krüger, Katrin Bekes, Hans-Guenter Schaller, Apical sealing ability of 2 epoxy resin–based sealers used with root canal obturation techniques based on warm gutta-percha compared to cold lateral condensation,

QUINTESSENCE INTERNATIONAL, VOLUME 38, NUMBER 3, MARCH 2007.

55. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. International endodontic journal. 2007 Nov 1;40(11):873-81.
56. Cohen: Pathways of the Pulp, 9th ed., Elsevier edition, Cv mosby,2006,page.no:351
57. Ajwani P, Saini N. The influence of the smear layer on dentinal tubule penetration depth by different root canal sealers-An in vitro SEM study. *J Endodontology* 2010; 22: 19-26.
58. Onay EO, Orucoglu H, Kiremitci A, Korkmaz Y, Berk G. Effect of Er, Cr: YSGG laser irradiation on the apical sealing ability of AH Plus/gutta -percha and Hybrid Root Seal/Resilon Combinations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology*. 2010; 110: 657-664.
59. Raid F. Salman, Ihsan N. Behnam et al ,Shear Bond Strength Measurement of Three Different Adhesive Sealers to Dentin & Gutta-percha (In Vitro Study), Zanco J. Med. Sci., Vol. 14,(Special issue 1), 2010.
60. Bernardes RA, De Amorim Campelo A, Junior DS, Pereiro LO, Duarte MA, Moraes IG, Bramante CM. Evalution of the flow rate of 3 nedodntic sealers: Sealer 26, AH Plus and MTA Obtura. Oral Surgery, Oral Medicine, Oral Pathology, Oral radiology, and Endodontology. 2010 Jan 31;109(1):47-9.

61. Balguerie E, van der Sluis L, Vallaeys K, Gurgel -Georgelin M, Diemer F. Sealer penetration and adaptation in the dentinal tubules: a scanning electron microscopic study. *Journal of endodontics*. 2011; 37:1576-9.

62. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. International endodontic journal. 2011 Jun 1;44(6):491-8 .

63. Kumar SA, Shivanna V, Naian MT, Shivamurthy G**.** Comparative evaluation of the apical sealing ability and adaptation to dentine of three resin-based sealers: An in vitro study**.** J Conserv Dent 2011 Jan 14(1):16-20.

64. Noushin shokouhinejad, mohammed Subeti, Hedayat Gorjestani, Mohammad Ali Saghiri, Mehrdad Lofti, Atefeh Hoseini. Penetration of Epiphany, Epiphany self-etch, and AH Plus into dentinal tubules: A scanning electron microscopy study. JOE 2011;37:1316-1319.

65. Gabriela alexandra Marín-bauza1, Yara teresinha correa silva-sousa , Suely aparecida da cunha, Fuad jacob abi rached-junior, Idomeo bonetti-filho, Manoel damião sousa-neto, Car63los eduardo saraiva miranda, Physicochemical properties of endodontic sealers of different bases, J Appl Oral Sci. 2012;20(4):455-61

66. Rupali Chadha, Sonali Taneja, Mohit Kumar, Sumit Gupta, An in vitro comparative evaluation of depth of tubular penetration of

three resin-based root canal sealers, Journal of Conservative Dentistry | Jan-Mar 2012 | Vol 15 | Issue 1)

67. Chandra Vijay Singh, S Anitha Rao, V Chandrashekar. An in vitro comparison of penetration depth of two root canal sealers: An SEM study. J Conserv Dent. 2012;15(3):261-264.
68. Joseph R, Singh S. Evaluation of apical sealing ability of four different sealers using centrifuging dye penetration method: an in vitro study. J Contemp Dent Pract. 2012 Nov 1;13(6): 830-3.
69. Nassar M, Hiraishi N, Islam MS, Aizawa M, Tamura Y, Otsuki M, et al. Effect of phytic acid used as etchant on bond strength, smear layer, and pulpal cells. Eur J Oral Sci 2013;121:482–7.
70. Aranda Garcia AJ, Kuga MC, Vitorino KR, Chavez-Andrade GM, Hungaro Duarte MA, Bonetti-Filho I, Faria G, Reis So MV. Effect of the root canal final rinse protocols on the debris and smear layer removal and on the push-out bond strength of an epoxy-based sealer. Microscopy research and technique. 2013 May 1;76(5):533-7.
71. Muliyar S, Shameem KA, Thankachan RP, Francis PG, Jayapalan CS, Hafi KA. Microleakage in endodontics. Journal of International Oral Health. 2014 Nov 1;6(6):99.
72. Ebert J, Holzschuh B, Frankenberger R, Petschelt A, Roggendorf MJ. Sealing ability of different versions of GuttaFlow2 in comparison to GuttaFlow and AH Plus. RSBO (Online). 2014 Sep;11(3):224-9

73. Daniela Kok, Ricardo Abreu Da Rosa, Mirela Sangoi Barreto, Fernanda Hoffmann Busanello, Manuela Favarin Santini, Jefferson Ricardo Pereira, Marcus ViníCius Reis Só Penetrability of AH plus and MTA fillapex after endodontic treatment and retreatment: A confocal laser scanning microscopy study. J Microscopy research and Technique. 2014;77(6):467-471.
74. Hengameh Ashraf,, Moha*mmad Asnaash*ari, Soheila Darmiani, Reza Birang.. Smear layer removal in the apical third of root canals by two chelating agents and laser: A comparative in vitro study Iraninan endo J 2014;9(3):210-214.
75. Nassar M, Hiraishi N, Tamura Y, Otsuki M, Aoki K, Tagami J. Phytic Acid: An Alternative Root Canal Chelating Agent. J Endod 2015; 41(2): 242-7.
76. Kong K, Islam MS, Nassar M, Hiraishi N, Otsuki M, Yiu CKY, et al. Effect of phytic acid etchant on the structural stability of demineralized dentine and dentine bonding. J Mech Behav Biomed Mater 2015;48:145–52.
77. Rogério Vieira Silva, Frank Ferreira Silveira, Martinho Campolina Rebello Horta, Marco Antônio Húngaro Duarte, Bruno Cavalini Cavenago, Ivaldo Gomes de Morais, Eduardo Nunes, Filling Effectiveness and Dentinal Penetration of Endodontic Sealers: A Stereo and Confocal Laser Scanning Microscopy Study, Brazilian Dental Journal (2015) 26(5): 541-546.

78. Rania Nassar, Mohannad Nassar. Antimicrobial effect of phytic acid on Enterococcus faecalis. The international Arabic Journal Of Antimicrobial Agents. 2016;6
79. Vineet Nikhil, Shikha Jaiswal, Parul Bansal, Rohit Arora, Shalya Raj, Pulkit Malhotra. Journal of Conservative Dentistry. 2016;19:179-183
80. Khader MA. An in vitro scanning electron microscopic study to evaluate the Dentinal Penetration Depth of three root canal sealers. Journal of International Oral Health. 2016 Feb1;8(2):191.
81. Merve Akcay, Hakan Arslan, Nazli Durmus, Merve Mese, and Ismail Davut Capar, Dentinal Tubule Penetration of AH Plus, iRoot SP, MTA Fillapex, and GuttaFlow Bioseal Root Canal Sealers After Different Final Irrigation Procedures: A Confocal Microscopic Study, Lasers in Surgery and Medicine 48:70–76 (2016).
82. Kong K, H, Nassar M, Hiraishi N, Otsuki M, Yiu CKY, et al. effect of phytic acid on resin-dentin bonding: Monamer penetration and stability of dentin collagen J of Prosthodontic Research 2016;
83. Saketh Puvvuda, Prasanna Latha, Jayalakshmi KB, Arul Selvan K. Comparative assessment of chelating and antimicrobial efficacy of phytic acid alone and in combination with other irrigants. International J Of Applied Dental Sciences 2017;3(2):19-22.

84. Grossman's Endodntic Practice. 13th Edition 2014, Chapter 15, Wolters Kluver, Pg no 367.

85. De Deus GA, Gurgel-Filho ED, Maniglia-Ferreira C, Coutinho-Filho T. Intratubular penetration of root canal sealers. Pesq Odonto Bras 2002;16:332–6.

86. De Deus GA, Gurgel-Filho ED, Maniglia-Ferreira C, Coutinho-Filho T. The influence of filling technique on depth of tubule penetration by root canal sealer: a study using light microscopy and digital image processing. Aust Endod J 2004;30:23–8.

87. Weis MV, Parashos P, Messer HH. Effect of obturation technique on sealer cement thickness and dentinal tubule penetration. Int Endod J 2004;37:653–63.

88. Ørstavik D. Materials used for root canal obturation: technical, biological and clinical testing.o Endod Topics 2005;12:25–38.

89. Lester KS, Boyde A (1977) Scanning electron microscopy of instrumented, irrigated and filled root canals. British Dental Journal 143, 359–67.

90. Sudha R, SukumaranVR, Ranganathan J, Bharadwaj N. comparative evaluation of the effect of two different concentrations of EDTA at two different of EDTA at two different pH and time periods on root dentin. J Cons Dent 2006;9:36-42.

91. Collet, R., Massilamoni, M., Kettering, J., Torabinejad, M., 1981. The biocompatibility of some root canal medicaments and irrigants. Int Endod. J. 14, 115–120.

92. Segura JS, Calvo JR, Guerrero JM, Llamas R. The disodium salt of EDTA inhibits the binding of vasoactive intestinal peptide to macrophage membranes: Endodontic implications. J Of Endod. 1996;22(7):337-340

93. Calt S, Serper A. Dentinal tubule penetration of root canal sealers after root canal+ dressing with calcium hydroxide. J Endod 1999;25:431–3.

94. Katoaka H, Yoshioka T, Suda H, Imai Y. Dentin bonding and sealing ability of a new root canal sealer. J Endod 2000;26: 230–5.

95. Vassiliadis LP, Sklavounos SA, Stavrianos CK. Depth of penetration appearance of Grossman sealer in the dentinal tubules: an in vivo study. J Endod 1994;20:373–6.

INSTITUTIONAL ETHICS COMMITTEE AND REVIEW BOARD

ADHIPARASAKTHI DENTAL COLLEGE AND HOSPITAL

Melmaruvathur, Tamilnadu-603019

An ISO 9001:2008 certified institution. Accredited by NAAC with "B" grade.

Recognised by DCI, New Delhi. Affiliated to: The Tamil Nadu Dr. M.G.R. Medical University, Chennai.

This ethical committee has undergone the research protocol submitted by **Dr. S. SARAVANAN** Post Graduate Student, Department of Conservative Dentistry and Endodontics under the title **"COMPARATIVE EVALUATION OF SEALER PENETRATION INTO ROOT DENTIN FOLLOWING PRETREATMENT WITH TWO DIFFERENT CHELATING AGENTS – AN INVITRO STUDY"** Reference No: 2015- MD-BrIV-SAT-10/APDCH under the guidance of **Dr. S. THILLAINAYAGAM MDS.,** for consideration of approval to proceed with the study.

This committee has discussed about the material being involved with the study, the qualification of the investigator, the present norms and recommendation from the Clinical Research scientific body and comes to a conclusion that this research protocol fulfils the specific requirements and the committee authorizes the proposal.

Date: **Member secretary**